

# **Improving ostrich welfare by developing positive human-animal interactions**

by

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In memory of Ndabenhle Eugene Mathenjwa  
“The General”

## **Declaration**

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the authorship owner thereof (unless to the extent explicitly otherwise stated) and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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## Abstract

Animal welfare has recently gained significant attention in commercial livestock industries worldwide. Specifically, several studies involving husbandry practices with positive human-animal interactions have shown a favourable link between improved animal welfare and production. However, limited research is currently available on optimal husbandry practices for the ostrich industry, which is still plagued by low fertility, high embryo and chick mortality, as well as variable growth rates. The poor ostrich production performance observed could thus reflect the difficulties of the birds to adapt to the commercial farming systems, and/or a failure of commercial practices to provide the basic requirements for this newly domesticated species. Hence, this study examined the effect of different husbandry practices varying in the intensity of human presence and interactions with ostrich chicks from day-old to 3 months of age on: weight gain, survival, immune competence, short- and long-term stress responses, social behaviour, docility, fear responses, meat quality and skin damage. The reproduction performance when the birds reached sexual maturity also was recorded.

The study showed that exposure to additional human contact (as compared to standard husbandry practices where human contact was limited to the provision of food and water), resulted in improved early growth, survival and immune competence, and even more so when chicks were exposed to gentle physical handling interactions. Furthermore, chicks exposed to such human presence and interactions expressed lower short-term stress responses when exposed to a potentially stressful event (i.e. feather harvesting and feather clipping), and lower long-term stress responses (as measured in corticosterone levels in the floss feathers), compared to chicks exposed to limited human presence. These results suggest an improved ability of the former to adapt to routine ostrich farm management practices. Furthermore, while social behaviour, docility and fear responses to humans when the birds reached the juvenile stage did not vary between the different husbandry practices, the birds habituated to

human presence at an early age were more likely to associate with a familiar rather than an unfamiliar human. This suggests that ostriches can not only discriminate between people, but also adjust their behaviour accordingly. Husbandry practices did not affect meat pH, meat colour, carcass attributes or skin damage. Females that received additional human presence along with physical gentle handling produced a higher number of eggs in their first year of reproduction as 2-year-olds, compared to females that were exposed to limited human presence. The difference in egg production was not observed during the second year of breeding. Hence, additional human presence combined with gentle physical handling seemed to improve early egg production and adaptation of females to the breeding environment.

The results of this study revealed that positive human-chick interactions could potentially alleviate problems associated with ostrich chick rearing, by reducing stress sensitivity while improving production performance. More specifically, this study has highlighted promising ways to overcome major constraints faced by ostrich farmers. A slight change of current management practices, through increasing positive human-animal interactions with these relatively wild animals has shown to be beneficial. However, further studies are required to assess the financial viability of these alternative husbandry systems. This will entail promoting animal welfare by comparing economic costs associated with different levels of animal welfare and the associated production performance in these production systems.

## Opsomming

Daar word tans wêreldwyd baie aandag aan diere welsyn by kommersiële plaasdiere geskenk. Verskeie studies het in hierdie verband op 'n gunstige verwantskap van positiewe mens-dierverhoudings met welsyn en produksie gedui. Daar is tans egter slegs beperkte navorsing beskikbaar sover dit optimale boerderytegnieke vir volstruise aangaan, terwyl die bedryf nog gebuk gaan onder lae vrugbaarheid, hoë embrionale- en kuikenvrektes, sowel as groot variasie in groeitempo. Hierdie swak prestasie hou moontlik verband met die onvermoë van die diere om by die kommersiële produksiestelsel aan te pas, en / of die onvermoë van die bedryfstelsels om aan die basiese behoeftes van hierdie onlangs gedomestikeerde spesie te voldoen. Hierdie studie het gevolglik die invloed van verskillende bestuurspraktyke wat verskil na gelang van die intensiteit van menslike teenwoordigheid en interaksie met volstruiskuike van dagoud tot 3-maande-ouderdom bestudeer ten opsigte van gewigstoename, oorlewing, immuunbevoegdheid, kort- en langtermyn stresreaksies, sosiale gedrag, makheid, vrees vir mense, vleisgehalte, velskade sowel as die reproduksieprestasie nadat geslagsrypheid bereik is.

Die studie het daarop gedui dat bykomstige kontak met mense (in vergelyking met die standaard bestuurspraktyk waar menslike blootstelling beperk was tot die verskaffing van voer en water) tot verbeterde vroeë groei, oorlewing en immuunbevoegdheid gelei het. Die effek is versterk waar dit met positiewe fisiese aanraking gepaard gegaan het. Kuikens wat aan intensiewe menslike teenwoordigheid gepaardgaande met positiewe fisiese aanraking onderwerp is, het laer vlakke van korttermynstres ervaar wanneer hulle blootgestel was aan potensieel stresvolle aktiwiteite (soos die oes en knip van vere). Voëls het ook laer langtermynstresvlakke gehad (soos gemeet met kortikosteroonvlakke in die vlosvere), in vergelyking met kuikens wat beperkte menslike blootstelling gehad het. Hierdie resultaat dui op 'n beter aanpassingsvermoë van die eersgenoemde groep by roetine

plaasbestuurspraktyke. Alhoewel sosiale gedrag, makheid en die vreesreaksies op mense by jong volstruise nie verskil het tussen die onderskeie behandelings nie, het die volstruise wat op 'n jong ouderdom gewoon geword is aan die menslike teenwoordigheid, meer geredelik met 'n bekende persoon as met 'n onbekende persoon geïnterreegeer. Dit suggereer dat volstruise dus nie net tussen mense kan onderskei nie, maar ook hulle gedrag ooreenkomstig aanpas. Bestuurspraktyke het nie vleis-pH, -kleur, karkaseienskappe of velskade beïnvloed nie. Wyfies wat as jong kuikens aan intensiewe menslike teenwoordigheid met positiewe fisiese hantering blootgestel is, het meer eiers tydens hulle eerste jaar in die broeikudde geproduseer as wyfies wat as jong kuikens blootgestel was aan beperkte menslike teenwoordigheid. Alhoewel hierdie verskil in eierproduksie nie tydens die tweede reproduksiejaar herhaal is nie, dui hierdie resultate daarop dat addisionele menslike teenwoordigheid gepaardgaande met positiewe aanraking na alle waarskynlikheid die aanvanklike aanpassing van wyfies by die broeiomgewing verbeter het.

Die resultate van hierdie studie dui daarop dat positiewe mens-kuikeninteraksies moontlik probleme wat algemeen met kuikengrootmaak ondervind word verminder kan word deur moontlik deur die vatbaarheid van die kuikens vir stres te verlaag, en so produksieprestasie kan verbeter. Meer spesifiek het die studie belowende maniere uitgewys waarvolgens die wesenlike beperkinge wat volstruisprodusente ondervind deur geringe aanpassings in die bestuurspraktyke wat tans in gebruik is, oorkom kan word. Verdere studies word egter benodig om die finansiële haalbaarheid van hierdie alternatiewe produksiepraktyke wat dierewelsyn voorop stel te ondersoek deur die ekonomiese kostes van die verskillende produksiestelsels wat verband hou met verskillende vlakke van dierewelsyn en die geassosieerde produksieprestasie van hierdie produksiestelsels te vergelyk.

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# **Chapter 1**

## **General Introduction**

Commercial ostrich farming originated in South Africa during the mid-19<sup>th</sup> century before spreading to other countries worldwide (Douglass, 1881; Smit, 1964). Initially, ostriches were mainly farmed in large numbers for their feathers (Douglass, 1881), until the sudden collapse of the feather trade between 1914 and 1945 (Dingle, 1996). After this recession the ostrich industry went through a complete restructuration and gained momentum in meat and leather trade which still thrive to date (Cloete et al., 1998). At present, meat and leather each contribute to about 45% of the total income from slaughtered birds, while feathers contribute the remaining 10% (Cloete et al., 2012). Leather derived from ostrich skin is currently competitive on international markets as a unique and attractive product (Al-Khalifa and Al-Naser, 2014). Furthermore, ostrich meat is gaining attention and preference over other meat as a result of its leanness, low levels of cholesterol and high bio-availability of protein (Balog and Almeida Paz, 2007; Cloete et al., 2008). Although some farmers sell infertile eggs, fats, egg shells and live chicks, the contribution of these products towards the overall income of an ostrich enterprise is not meaningful (Bejaei and Cheng, 2014).

Despite some advancement, the production in the ostrich industry is still compromised by factors such as low fertility, poor hatchability of eggs, high chick mortality rates and the relatively wild behaviour of ostriches, making them difficult to handle and manage efficiently (Verwoerd et al., 1999). Mortality rates of ostrich chicks between hatching and 3 months of age may reach levels of up to 50% and higher under commercial farming conditions, making this period the most critical stage in the rearing of ostrich chicks (Verwoerd et al., 1999; Cloete et al., 2001). The occurrence of such high mortality rates is suggested to be the result of stress caused by the failure of the chicks to adapt to the rearing environment, diseases, malnutrition and poor management which collectively impair animal welfare and production (Mitchell, 1999; Verwoerd et al., 1999; Verwoerd, 2000; Cloete et al., 2001; Iji, 2005; Glatz and Miao, 2008).



Animal welfare has recently gained attention in several livestock industries (Mellor et al., 2009). The primary concern voiced by advocates of animal welfare is that farmed animals should receive freedom from hunger and thirst, freedom from discomfort, freedom from pain, injury or disease, freedom from fear and stress as well as the freedom to express the normal range of behaviour repertoires inherent to the species (Farm Animal Welfare Council, 2009). Moreover, the quality of life experienced by such animals should make it worthwhile living. However, this vision is not always achieved in commercial ostrich farming operations (Cloete and Malecki, 2011) and therefore requires further action/research. In other large-scale intensive livestock production units such as in the dairy, poultry and pork industries comprehensive research has been conducted where positive human-animal interactions have been integrated into husbandry practices to improve animal welfare and production (Hemsworth, 2003; Waiblinger et al., 2006; Zulkifli, 2013). Other studies have suggested that positive human-animal relationships alleviated stress in cattle and sheep and facilitated a better occupational environment for both livestock and the stockpeople caring for them (Hemsworth et al., 2011). In addition, positive regular gentle handling of chickens was positively correlated to growth, immune competence and egg production (Gross and Siegel, 1982, 1983; Barnett et al., 1994; Zulkifli et al., 2002), while reducing litter mortality rates in pigs (Rushen et al., 1999). Production parameters were also improved in dairy cows whereby positively handled heifers produced more milk with higher milk protein and more fat content than negatively handled heifers (Hemsworth et al., 2000).

While the renowned aggressive nature of particularly male ostriches may compromise the application of proper management and impair bird and handler welfare, some birds do display an interest to associate with humans (Malecki and Rybnik-Trzaskowska, 2011). Thus, it has been suggested that integrating extensive human presence and positive regular handling at an early age within the husbandry regime currently in place could improve the welfare of

ostriches. Preliminary studies have already revealed beneficial results of applying positive human-chick interactions at an early age in terms of improving growth and survival during the first few weeks post hatching and also improving the willingness of juvenile ostriches to associate with humans (Wang et al., 2012; Bonato et al., 2013). However, other researchers have argued that ostriches habituated to humans may have their production performances compromised as they would be more inclined to direct their sexual interest towards human than towards their mates (Bubier et al., 1998). Yet there is no information available to confirm or refute this argument, nor whether exposing chicks to positive human-bird interactions could improve the overall welfare of ostriches. Therefore, the aim of this study was to evaluate the effect of husbandry practices involving extensive human presence and gentle handling at an early age (0-3 months of age) on ostrich growth, survival, immune competence, stress responses, docility and reproduction performance when mated in pairs.

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# **Chapter 2**

## **Literature review**

## 2.1 The concept of human-animal interactions in farm animals

Humans and animals are in regular and at times in close contact in modern livestock industries (Hemsworth, 2003). The contact between humans and animals may be visual, tactile, olfactory and/or auditory and may vary from negative to positive in nature, and from frequent to infrequent in occurrence (Hemsworth, 2003; Waiblinger et al., 2006). Positive human-animal interactions manifest as gentle handling of animals (i.e. patting, stroking, brushing, general human presence, a caring and gentle attitude etc.), while negative interactions refer to rough and inappropriate handling practices (i.e. punishment, shouting, kicking, slaps, rapid movement among animals, etc.). Both types of interactions have been shown to affect animal welfare, management and productivity in a large range of livestock species (Hemsworth, 2003; Waiblinger et al., 2006; Hemsworth and Coleman, 2011). Hence, animals that have experienced positive interactions with humans may associate more easily and willingly with them. On the other hand negatively handled animals may be conditioned to such practices and later develop a fear of human handlers (Hemsworth, 2003; Hemsworth and Coleman, 2011). Therefore, an increase in the level of fear of humans may cause stress in animals and thus compromise management, production and ultimately welfare. Animal welfare in itself attracts growing concern of the public worldwide (Rushen et al., 1999; Hemsworth, 2003).

## 2.2 Human-animal interactions and animal welfare

Animal welfare has received considerable attention recently in all domestic livestock industries (Mellor et al., 2009). According to Hemsworth and Coleman (2011), animal welfare is a complex term that can be defined in many ways, depending on how it is measured. For instance, Broom (1986) defined animal welfare as the ability of an animal to entirely cope with its environment without any difficulties. However, this definition was



confined mainly to animals kept in an environment other than their natural habitat, as they may encounter more discomfort and stress in such an unfamiliar setting. Recently, the Farm Animal Welfare Council (2009) defined animal welfare, based on the five freedoms adopted for animals under the care of humanity, namely freedom from hunger and thirst, freedom from discomfort, freedom from pain, injury and disease, freedom to express normal behaviour and freedom from fear and/or stress. Hence, the disregard of any of these freedoms may lead to serious biological incidences such as immune suppression, increased disease infection, increased mortality, poor growth and reproduction efficiency. As a consequence of stress, it may indicate that animal welfare is compromised (Broom, 1986). More recently, Glatz (2011) introduced to the concept of animal welfare, the obligation to supply commendable husbandry practices. This includes combining good animal health (by providing good nutrition and early detection/prevention of diseases) with positive human-animal interactions.

Subsequently, there has been a growing interest in optimising animal welfare by improving husbandry practices and applying proper animal management practices (Blokhuys, 2005; Glatz and Miao, 2008). The application of positive human-animal interactions then became one of the most desirable interventions to improve farm animal welfare (Hemsworth and Coleman, 2011). Such interventions could potentially reduce the fear of humans by animals, commonly observed in farming environments. Fearfulness in animals (whether caused by humans or specific environmental conditions or management procedures) typically triggers stress response mechanisms (Hemsworth, 2003), which could be used to assess the state of animal welfare (Broom, 1986; Sejian et al., 2011).

### 2.2.1 Stress response mechanisms: the influence on physiological and behavioural responses

Stress is of great concern regarding animal welfare when animals have to make extreme and sometimes prolonged physiological and behavioural adjustments to cope with changes in their environment (Kumar et al., 2012). In some severe cases, vital indicators of general well-being such as growth, survival or immune competence can be compromised by stressful episodes. This may result in failure of animals to express their full genetic potential at best to distress, or death at the very worst (Gross and Siegel, 1983a; Broom, 1986).

#### *2.2.1.1 The effect of stress on physiological responses*

When animals are exposed to stressful events, they undergo physiological changes involving the activation of the sympathetic-adrenal-medullary axis (SAM) and the hypothalamic-pituitary-adrenal axis (HPA), controlled by the hypothalamus (Rhodes et al., 2009). The SAM and the HPA axes are two well-known physiological stress coping mechanisms of animals (Hemsworth and Coleman, 2011). The activation of the SAM axis following exposure to a stressful event leads to the rapid secretion of catecholamine hormones (i.e. adrenaline, noradrenaline) into the bloodstream. These hormones stimulate the physiological changes preparing the body of the animal for physical action such as fight or flight response (McCarty et al., 1988; Hemsworth and Coleman, 2011). If the exposure to the stressful episode persists, the HPA axis (second element of stress responses) is activated (Hemsworth and Coleman, 2011). The HPA axis leads to the secretion of glucocorticoid hormones; cortisol in mammals and corticosterone in birds (Rhodes et al., 2009). These glucocorticoid hormones have glucose-regulating properties which also influence many other metabolic functions in a number of tissues. As such, an increase of the concentration of these hormones may depress vital functions such as the ability to fight parasites and pathogens (i.e. immune

competence), which could in turn compromise weight gain, survival and reproductive performance (Stephenson, 1994; Hemsworth and Coleman, 2011).

For instance, studies in pigs (Hemsworth et al., 1981), dairy cows (Hemsworth et al., 1989; 2000; Breuer et al., 2003) and sheep (Tosi and Hemsworth, 2002) all revealed that animals that received less attention from humans or were handled in a negative way showed an increase in indicators of physiological stress during handling. Chickens exposed to negative interactions with humans had an increase in Heterophil/Lymphocyte ratio (H/L ratio a good physiological indicator of stress in animals) and poor immune competence as indicated by a low antibody response and higher susceptibility to infections by *Escherichia coli* bacteria (Gross and Siegel, 1982; 1983a; 1983b; Hemsworth et al., 1994; Zulkifli et al., 2002; Zulkifli and Siti Nor Azah, 2004). Consequently, exposure to long-term or frequent stressful events is likely to reduce the animal's fitness, which can be expressed through failure to achieve production performance standards or lead to disease and in severe cases distress and eventual death.

#### *2.2.1.2 Behavioural responses to farm management procedures: the effect of fear and stress when handling*

Although the majority of farm animals are considered domesticated, some animals still show a regular/constant fear of humans as reflected by their avoidance response towards humans (Rushen et al., 1999; Waiblinger et al., 2006). Such negative behavioural responses severely constrain the ease of handling and management leading to frustration and, occasionally, to abuse by human handlers. Moreover, fearful animals may be dangerous to themselves and to their handlers during farm management procedures (Rushen et al., 1999). Several behavioural tests have been developed to assess fear responses of farm animals towards handlers (Grignard et al., 2001; Waiblinger et al., 2006; Forkman et al., 2007; Mazurek et al., 2011).

These tests evaluate well-defined animal characteristics such as approach/avoidance of humans, aggressiveness or the lack thereof as well as docility (Hemsworth et al., 1994; Grignard et al., 2001; Zulkifli et al., 2002; Breuer et al., 2003; Zulkifli and Siti Nor Azah, 2004; Waiblinger et al., 2006; Forkman et al., 2007; Mazurek et al., 2011). The rationale is that fearful animals not accustomed to human presence will consistently avoid them, display aggressive or flight behaviours and will be difficult to handle during the tests (Waiblinger et al., 2006). In contrast, less fearful animals habituated to humans will be characterised by a lack of human avoidance or even willingness to associate with humans, a sociable demeanour as well as a lack of aggression towards handlers.

The principle of habituating animals to positive interactions with humans has been proven in several studies conducted on poultry (Jones and Waddington, 1992; Barnett et al., 1994; Zulkifli and Siti Nor Azah, 2004), cattle (Munksgaard et al., 1999; Rushen et al., 1999; Lensink et al., 2000; Breuer et al., 2003), and pigs (Hemsworth et al., 1994; Tanida and Nagano, 1998). It is contemplated that habituation will reduce fear in animals and facilitate proper and caring animal management (Hemsworth and Coleman, 2011). For instance, dairy cows habituated to positive human contact through gentle brushing were easier to handle during the milking process (and showed a lack of vocalization, as well as a normal heart rate during milking) as compared to cows habituated to negative human treatment (Rushen et al., 2001; Bertenshaw et al., 2008). These results suggest that positive interactions may play a comforting role in dairy cows during the milking phase. Furthermore, the ability of animals to distinguish between positive and negative handlers has been adequately demonstrated in dairy cows (Munksgaard et al., 1999) and pigs (Tanida and Nagano, 1998; Koba and Tanida, 2001). Such differentiation by animals suggests that the ease of handling of animals during routine farm procedures is likely to be influenced by their former experience with specific

humans and emphasise the long-term benefits of developing good relationships between stockpersons and animals.

## 2.3 Human-animal interactions and productivity

### 2.3.1 Effects on quantitative production traits

Productivity of several livestock species has been influenced by the relationship between humans and their animals (Rushen et al., 1999; Hemsworth et al., 2011). Impaired growth, poor reproduction, a reduced milk yield and decreased egg production were observed in a wide variety of species in response to poor, indifferent and/or negative handling (Sejian et al., 2011). Research with experimental and commercial pigs have shown that high levels of fear for humans may cause chronic stress responses or a series of acute stress responses upon exposure to humans which may severely depress an animal's production level (Hemsworth et al., 1981, 1986, 1987; Gonyou et al., 1986). Therefore, animals that are handled in a negative way are likely to exhibit fear of humans, which initiate stress responses mechanisms and the reallocation of resources originally earmarked for production (Gross and Siegel, 1982; Hemsworth, 2007). On one hand, the negative handling of dairy cows inhibited the milk let-down mechanism and increased the residual milk volume (Bertenshaw et al., 2008). On the other hand, pigs that were positively handled had an improved growth rate, a reduced number of days to first oestrus, improved pregnancy rate and a reduced litter mortality rate (Rushen et al., 1999). Moreover, positive human-animal interactions in poultry resulted in higher weight gains (Gross and Siegel, 1982; Zulkifli and Siti Nor Azah, 2004), and an improved egg production (Barnett et al., 1994), compared to animals exposed to negative or limited human contact.

### 2.3.2 Effects on qualitative production traits

Poor husbandry practices such as inappropriate handling, improper use of sticks or prods by handlers, violent impact of the animals against facilities or impact with other animals are potential bruising events which could reduce product quality (Warriss, 1990). This is specifically true prior to slaughter, as animals are exposed to all kinds of stressors, ranging from physical challenges (i.e high ambient temperatures, confinement, handling, noise, crowding etc.) to psychological restrictions (i.e breakdown of social structure, mixing with unfamiliar animals, unfamiliar or noxious smells and a novel environment; Warriss, 2000; Chulayo et al., 2012). The increase in the levels of physiological stress responses as a result of all these factors (Hemsworth, 2003; Waiblinger et al., 2006) deplete muscle glycogen concentration affecting the meat pH of slaughter animals (Warriss, 1990; Chulayo et al., 2012; Adzitey, 2011). Moreover, bruises resulting from injuries during transportation or handling are known to decrease the commercial value of meat and hence lead to financial losses for farmers (Grandin, 1980). However, positive human-animals interactions and gentle handling may counteract poor product quality through reducing fear of humans, which leads to stress being alleviated. Sheep, cattle and veal calves handled gently displayed lower levels of stress during handling in the abattoir with a potential beneficial effect on meat quality (Lensink et al., 2001; Hemsworth et al., 2011). According to Fordyce et al. (1988), beef cattle exhibiting stress responses such as frequent vocalising during handling when restrained produced tougher meat. Similarly, an increase in physiological stress responses resulted in reduced milk fat and protein concentrations in dairy cows (Hemsworth et al., 2000), emphasizing the importance of ensuring positive human-animal interactions.

## 2.4 The ostrich industry in relation to modern agricultural practices

### 2.4.1 Biological description of the ostrich

The ostrich (*Struthio camelus*), is the largest living bird and a member of the ratite group, also known as flightless birds due to the lack of a keel from the sternum (Hermes, 1996; Deeming, 1999). The other members of the ratite family include the emu (*Dromaius novaehollandiae*), the cassowary (*Casuarius casuarius johnsonii*), the greater and lesser rhea (*Rhea americana* and *Rhea pennata* respectively) and the kiwi (*Apteryx* spp.). At present, there are only four extant sub-species of ostriches namely; *S. c. camelus* (the Arabian ostrich), *S. c. molybdophanes* (the Ethiopian ostrich), *S. c. massaicus* (the Kenyan redneck ostrich) and *S. c. australis* (the southern ostrich strain, sometimes referred to as the Zimbabwean Blue ostrich) (Deeming, 1999). A sub-species known as the *S. c. syriacus* once occurred in the Syrian and Arabian region, but was hunted to extinction (Deeming, 1999). The domestic race farmed within South Africa is considered as a hybrid of *S. c. camelus* and *S. c. australis* and is sometimes denoted as the strain *S. c. domesticus* (Swart, 1988).

Matured ostriches are sexually dimorphic; males have a jet-black plumage with white feathers, pink beak and shins. Females have a dull brown-grey plumage with white feathers, dark-grey beak and shins (Deeming, 1999). Ostrich chicks and juveniles can only be sexed on the basis of their physical appearance at the age of 12-14 months (Mine et al., 2002). At sexual maturity (approximately 2 years of age), ostriches can grow to above 2 metres and weigh more than 150 kilograms (Deeming, 1999). Ostrich breeding in the wild or flock mating system is considered complex, as both males and females have multiple partners. Furthermore, the communal nesting system of ostriches is unique in the sense that although females lay eggs collectively in multiple nests, only the major male and female (the pair that established the nest) incubate the eggs and guard the chicks until their independence at 12

months of age (Deeming and Buiber, 1999; Kimwele and Graves, 2003). The major male usually incubates the eggs at night with the major female incubating during daylight hours until hatching at  $\pm 42$  days (Deeming and Bubier 1999; Kimwele and Graves, 2003).

#### 2.4.2 History of ostrich farming

Ostrich farming originally started in the mid-19<sup>th</sup> century when ostriches were mainly farmed in large numbers for their feathers in the Klein Karoo region of South Africa (Douglass, 1881, Smit, 1964). In 1913, feathers derived from ostriches were among the top 4 largest exports from South Africa following gold, diamonds and wool derived from sheep (Jorgensen, 2014). However, the onset of World War I in 1914 resulted in the collapse of the feather trade with the majority of ostrich farmers during this time facing a major financial crisis (Jorgensen, 2014).

The ostrich industry of South Africa regained momentum in the 1940s by the introduction of meat and leather derived from ostriches as the products of choice (Jorgensen, 2014). Other countries such as Israel, USA and Australia also adopted ostrich farming as a novel industry in their agricultural production system at that stage. Between 1964 and 1970 the first ostrich abattoir and tannery were established in Oudtshoorn (South Africa) by the Klein Karoo Landbou Koöperasie. An abattoir accredited by the European Union was opened for the international trading of ostrich meat during 1993. However, the recurrent outbreaks of highly pathogenic avian influenza in South Africa since 2004 has currently threatened the success of this once-thriving industry as successive bans on the export of ostrich meat were implemented (Jorgensen, 2014).



### 2.4.3 Current challenges in ostrich farming

Although considerable progress has been achieved in improving ostrich production, the industry is still plagued by factors such as poor chick survival, variable chick growth and diseases (Verwoerd et al., 1999; Verwoerd, 2000; Cloete et al., 2001). Mortality in ostrich chicks before the age of 3 months old can reach up to 50% or above, on commercial farms (Verwoerd et al., 1999; Cloete et al., 2001). Such high mortality rates may be attributed to the susceptibility to diseases, stress and poor management practices (Verwoerd, 2000; Cloete et al., 2001; Glatz and Miao, 2008). Furthermore, as the ostrich industry is relatively unique, the requirements of this species under farming conditions are still not well understood (Deeming, 2011). Thus, the abysmal levels of rearing failure and variable growth encountered by the ostrich industry could reflect the failure of the chicks to adapt to husbandry practices currently in place. Also, this could reflect a general failure of the industry to provide husbandry practices within the adaptive limits of ostrich chicks.

### 2.4.4 The rearing of ostrich chicks in modern farming practices

Two common methods are currently used for rearing ostrich chicks, namely; foster rearing and intensive rearing methods (Verwoerd et al., 1999). The foster rearing method (also referred as the adoption method) involves the rearing of chicks by a surrogate breeding pair (or “foster parents”). It is commonly practiced in areas where irrigated pastures are available to limit feeding cost (Verwoerd et al., 1999). The breeding pair used as foster parents hatch at least one egg of their own before additional chicks from artificially incubated eggs are added to the brood (Wang et al., 2012). Depending on the number of chicks allocated to the foster parents, a simple infrastructure may be needed to keep chicks safe from inclement weather (Verwoerd et al., 1999; Wang et al., 2012). Wang et al. (2012) demonstrated that chicks reared using foster parenting grew faster and survived better than chicks reared by

conventional rearing methods where human intervention was limited to the provision of food and water. However, it was also shown that foster parent pairs varied markedly in their rearing success (Wang et al., 2012). Moreover, chicks reared using this method showed appreciably less interest to associate with humans (Bonato et al., 2013) and were more difficult to handle (Bonato, Pers. Communication).

The intensive rearing method is perhaps the most widely used method in the ostrich industry and involves keeping chicks indoors after hatching for at least a week. The chicks are restricted access to the outside environment and supplied with feed and fresh water (Verwoerd et al., 1999; Bunter, 2002). Temperatures in such chick rearing facilities are commonly controlled by the use of automatic electronic heaters set at a temperature of approximately 30°C with a gradual decline of 0.5°C /day until a temperature of 26°C is reached (Verwoerd et al., 1999). Extremely low and high temperatures are usually avoided as they can respectively lead to hypothermia or hyperthermia, resulting in complications involving chick health. However, as the chicks grow they are gradually allowed access to the outside environment on lucerne camps on days of adequate weather and taken back to the rearing facility during the nights (Verwoerd et al., 1999; Bunter, 2002). This procedure is beneficial for intensively reared chicks, as it encourages exercise (Verwoerd et al., 1999; Glatz and Miao, 2008). The house flooring of the chick rearing facility is designed in such a way that it allows easy cleaning of faeces and urine to reduce the accumulation of bacteria (Verwoerd et al., 1999). Furthermore, the ventilation system should allow an easy flow of air to reduce excess accumulation of ammonia, which can negatively affect chick health and the occupational health and safety of staff (Glatz, 2011). Chicks generally stay in the chick-rearing infrastructure for up to 12-16 weeks before being transferred to a feedlot or an alternative growing-out facility (Verwoerd et al., 1999; Glatz and Miao, 2008; Deeming, 2011). The very nature of intensive chick rearing results in ostrich chicks reared by this

method to encounter a certain degree human presence, as compared to chicks reared by foster parents. Preliminary investigations on the effect of extensive human presence during the rearing phase has suggested potential benefits on early production traits of chicks (Wang et al., 2012), as well as favourable behavioural repertoires of juveniles (Bonato et al., 2013). The initial objective of extensive human presence as an intervention was to determine whether treated chicks would be more likely to display a behaviour that could facilitate artificial reproduction techniques in this species. However, based on the success of the treatment in improving early chick wellbeing, subsequent studies were designed to also consider this important aspect.

## 2.5 Aims of the study

Against the background of evidence that slight changes affected to the standard husbandry practices currently in place by deliberately introducing positive human-chick interactions at an early age in ostrich rearing was associated with biological gains, as motivated above, the following objectives were set for this study:

1. To confirm that survival and growth during the critical stage of life in ostrich chicks (0-3 months old; Wang et al., 2012) and docility later in life (12 months old; Bonato et al., 2013) would benefit from extensive human presence,
2. To determine whether immune competence, stress responses, as well as juvenile product quality and adult productivity of ostriches would also benefit from extensive human presence, as demonstrated in a wide variety of other domestic livestock species (poultry, cattle and pigs: Hemsworth et al., 1981, 1989, 2000; Gross and Siegel, 1983a, 1983b; Zulkifli et al., 2002; Breuer et al., 2003). Alternatively, it needed to be tested whether close involvement with humans at an early age, leading to

potential sexual interest of ostriches in humans, would compromise productivity under natural mating conditions, as was postulated by Bubier et al. (1998).

To achieve this, the effect of early habituation of ostrich chicks to human presence and gentle handling during their first 3 months of age was evaluated on survival, growth and immune competence in Chapter 3. This was achieved by recording and comparing weights and survival at 6 and 12 weeks old of chicks exposed to a different degree of human care and presence. The antibody responses of these chicks following Newcastle disease vaccination was then assessed when they reached 5 months of age. Secondly, the short- and long-term stress responses, social behavioural responses, fear and docility of these groups of chicks towards humans were evaluated in Chapter 4. In this chapter, feather harvesting and feather clipping procedures were used as short-term stress incentives followed by the measuring of the H/L ratio before and after the feather treatment. Long-term stress responses were measured by assessing the level of corticosterone accumulated in the chicks' feathers. Social behavioural responses were evaluated using the response of ostriches towards humans as described by Bonato et al. (2013), while fear and docility tests were performed using protocols adapted from other livestock species (Mazurek et al., 2011). Finally, the effect of habituating ostriches to extensive human care as chicks was assessed on important slaughter traits of economic importance, such as meat quality and skin damage in Chapter 5. The impact of extensive human care on adult reproduction traits of pair-bred females in the breeding flock over two breeding seasons was also assessed in this chapter using trait definitions supplied by Bunter (2002).

## 2.6 References

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## **Chapter 3**

# **Extensive human presence and regular gentle handling improves growth, survival and immune competence in ostrich chicks**

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## Abstract

Commercial ostrich farming is still hampered by poor chick performance, especially during the first three months post-hatching. Ostriches are prone to stress and alternative rearing methods that include extensive human presence and gentle physical interactions with the chicks may consequently be beneficial for crucial physiological variables in this sensitive period. The aim of this study was thus to evaluate the effect of human presence and human-chick interactions on the growth, survival and immune competence of ostrich chicks. A total of 416 day-old ostrich chicks of mixed sex and breed were randomly allocated to one of three different husbandry practices, namely; I1 (extensive human presence with audio stimuli and gentle physical interactions), I2 (extensive human presence with audio stimuli but without physical interactions) and S (human presence limited to supply of feed and water as control) during the 2013 and 2015 breeding seasons. The groups of chicks were exposed to one of these husbandry practices respectively, for three months and mixed together thereafter. Chick weight (kg) and survival (%) were measured when 6- and 12-weeks of age. Vaccination against Newcastle disease virus (NCD) was performed at 20 weeks of age and the chicks' antibody responses to the vaccine was measured using the Hemagglutination-Inhibition (HI) test. I1 chicks were heavier at 6 weeks of age than chicks in the two other groups (I1:  $7.47 \pm 0.18$  kg; I2:  $7.06 \pm 0.15$  kg and S:  $6.21 \pm 0.13$  kg;  $P < 0.05$ ). However, at 12 weeks of age the weight of the chicks did not vary between husbandry practices ( $P > 0.05$ ). Chick survival to 6 weeks of age was significantly higher for the I1 and I2 groups compared to the S group (I1:  $87.50 \pm 1.20$ %; I2:  $86.9 \pm 1.20$ % and S:  $83.70 \pm 1.30$ %;  $P < 0.05$ ). No difference in chick survival rates was recorded between husbandry practices at 12 weeks of age ( $P > 0.05$ ). A higher percentage of positive HI titers were measured for the S and I2 chicks following NCD vaccination, compared to I1 chicks. These suggest that I1 chicks had an improved immune competence. Positive human-chick interaction at an early age apparently improved the

performance of ostrich chicks and improved their immune competence. Integrating extensive human presence along with positive human-chick interactions may thus assist in alleviating problems related to early chick rearing in the ostrich industry.

### 3.1 Introduction

Ostrich farming has been part of the livelihood of farmers in the Klein Karoo region of South Africa for the past 150 years (Smit, 1964). Since then, the interest in ostrich farming increased worldwide following the high returns generated by trading of ostrich products. In comparison to other livestock industries, the ostrich industry is, however, still underdeveloped (Cloete et al., 2012). Previous studies have identified the inefficiency of the ostrich industry as mainly caused by factors such as a variable chick growth, poor chick survival and the susceptibility to diseases (Verwoerd et al., 1999; Verwoerd, 2000; Cloete et al., 2001; Bunter, 2002). Such limitations may stem from an inability of chicks to adapt to the existing husbandry practices (Siegel, 1984).

Husbandry practices are well known to markedly impact on animal welfare (Barnett and Newman, 1997). Inappropriate husbandry practices such as aversive handling, shouting, mistreating and ignoring animals have been demonstrated to compromise animal welfare and may impair the performance (Jones and Waddington, 1992; Hemsworth, 2003). Exposing young gilts to aversive handling has been shown to reduce the weight gains, compared to positively handled gilts of the same age (Hemsworth et al., 1981; Gonyou et al., 1986). Moreover, the growth rate of aversively handled pigs was reduced by 11%, while litter mortality was also increased (Rushen et al., 1999).

In contrast, husbandry practices involving regular gentle handling and extensive human presence improved animal welfare (Rushen et al., 1999; Hemsworth, 2003; Zulkifli, 2013). For example, chickens that were handled gently during rearing demonstrated

improved growth rates and feed conversion ratios (Jones and Hughes, 1981; Gvoryahu et al., 1989). Although certain researchers did not find differences in feed efficiency between regularly handled and ignored pullets, increased weight gains was reported in pullets exposed to regular gentle handling compared to those that were ignored (Collins and Siegel, 1987). Regular gentle handling in poultry also improved the immune status of birds, as indicated by an increased antibody production and an advanced resistance to *Escherichia coli* infection (Gross and Siegel, 1982).

Limited research on husbandry practices aimed at improving the welfare of ostrich chicks is currently available. The development of appropriate husbandry practices for ostrich chicks is thus still on-going, compromising the ability of farm staff to provide suitable conditions that will maximise their welfare (Deeming, 2011). Mortality rates of ostrich chicks before three months old may reach up to 50% or more, on commercial ostrich farms (Verwoerd et al., 1999; Cloete et al., 2001). Mortality of ostrich chicks post hatch have appeared to be influenced by parental and incubation factors (Cloete et al., 2001; Wang, 2012). While aspects like flock structure, incubation equipment and incubator management may be optimised, chick survival may still be improved further by applying appropriate husbandry practices (Verwoerd et al., 1999; Wang et al., 2012). Verwoerd et al. (1999) reported reduced mortality rates of about 10-15% under more intensively supervised husbandry conditions. More recently, Wang et al. (2012) reported survival rates reaching 97% up to 4 weeks of age in chicks that were reared under a husbandry practice involving extensive human presence and regular gentle handling. These results suggest that chick survival may be optimised by improving husbandry practices in the ostrich industry.

However, Wang et al. (2012) did not evaluate the immune competence of ostrich chicks exposed to human presence. Vaccination of ostriches against the Newcastle disease virus is a standard protocol for South African ostrich farmers to prevent Newcastle disease



outbreaks and to reduce the risk of spreading the virus to other countries, as most ostrich meat produced is exported (Alexander, 2000; Blignault et al., 2000). From an animal welfare perspective, the antibody response to a vaccine may differ between chicks reared using different husbandry practices, which differ in human presence and care. This is because chicks reared under extensive human presence may experience less fear induced by early life rearing conditions (Zulkifli and Siti Nor Azah, 2004). Consequently, this could result in reduced levels of environmental stress compared to chicks exposed to limited human care. Zulkifli et al. (2002) demonstrated that extensive human presence improved antibody response of broiler chickens to Newcastle disease vaccine. No studies have investigated similar treatments in ostriches. Therefore, the aim of this study was to evaluate whether extensive human presence along with positive human-chick interactions improved growth, survival and immune competence in ostrich chicks under commercial farming conditions.

### 3.2 Materials and methods

#### 3.2.1 Sampling population and study area

The experiment was conducted at the Oudtshoorn Research Farm of the Western Cape Department of Agriculture from August 2013 to November 2013, and repeated from September 2015 to December 2015. The chicks were offspring of the farm's breeding pairs whose management had been previously described (Bunter and Cloete, 2004; Cloete et al., 2008). Day-old chicks were obtained from combinations of pure-bred parents namely, South African Blacks (SAB), Zimbabwean Blues (ZB) and Kenyan Reds (KR), or a combination of crosses involving these strains. Chicks from cross-bred combinations were grouped into two categories: crosses between ZB and SAB parents, and crosses between KR and SAB parents, to facilitate the analysis as some cross-bred combinations were only represented by very few chicks. Pooling led to 5 breeds used for statistical analysis namely; SAB, ZB, KR, SAB×ZB

and SAB×KR. Ethical clearance to conduct this study was granted by the Departmental Ethical Committee for Research on Animals of the Western Cape Department of Agriculture (Ref No.: R13/81).

### 3.2.2 Husbandry practices

A total of 416-day-old chicks were randomly allocated to three husbandry practices: standard husbandry practice (S, N=66 and 70 for 2013 and 2015 respectively); additional human presence (as compared to S) involving regular physical contact, audio and visual stimuli (I1, N=68 and 76 for 2013 and 2015 respectively), and additional human presence, audio and visual stimuli but no physical contact (I2, N=66 and 70 for 2013 and 2015 respectively). The S husbandry practice followed the standard protocol used at the Oudtshoorn Research Farm, with human contact with chicks limited to the provision of food and fresh water (Bunter, 2002). The I1 husbandry practice allowed chicks to be familiarized with human touch, human voices, handfeeding and a general human presence. As regular handling of every bird may not be practical on a commercial scale, chicks of the I2 husbandry practice were exposed to a similar intensive human presence and schedule than the I1 husbandry practice but without physical stimuli. These husbandry practices were carried out from day-old chicks to 12 weeks of age, as follow: for the first 7 days of the experiment (week 0: day 1 to day 7), human presence was provided to the I1 and I2 group of chicks for 100% of the daylight hours (6:00 to 18:00). From week 1 (day 8-14), chicks were visited for 1 hour at time intervals increasing weekly by 1 hour. Therefore, during week 2 (day 15-21), chicks were visited for 1 hour every 2 hours. Week 3 (day 22-28) chicks were visited for 1 hour every 3 hours etc. At week 8, chicks were only visited for 1 hour early in the morning (7:00 to 8:00) and during the afternoon (16:00-17:00), until week 12 when I1 and I2 husbandry practices were terminated. Hereafter all chicks were reared together in a single group, using the S husbandry practice.

The experimental chicks in all groups received the same commercial ostrich diet formulated at the farm and water *ad libitum* (Brand, 2014).

### 3.2.3 Chick weight, survival and immune competence

Chicks were individually marked after hatching and their gender determined through vent-sexing (Minnaar, 1998). The weight of each chick was measured at day-old, 6-weeks of age and 12-weeks of age. Survival was monitored from day-old to 12-weeks of age, and compared at 6 weeks and 12 weeks of age. When 20 weeks old, all the remaining chicks (N=341) were vaccinated against Newcastle disease (NCD) by injecting 1mL of STRUVAV ND PLUS (Deltamune PTY LTD) vaccine subcutaneously at the back of the neck. Blood samples were collected from the jugular vein in Vacutainer tubes prior to the injection, and 21 days post-injection. Blignault et al. (2000) demonstrated that 21 days post-injection corresponded to the period of peak antibody response expected for the vaccine. To assess the level of antibodies in the plasma, a Hemagglutination-Inhibition (HI) test was performed according to the recommendations of the OIE (2008). Briefly, 0.025mL of phosphate-buffered saline solution was dispensed into each well of a plastic V-bottomed microtitre plate. A volume of 0.025mL of serum was then placed into the first well of the plate. Two fold dilutions of 0.025mL volumes of the serum were made across the plate and 4 Hemagglutinin Units (4 HAU) of the NCD antigen added to each well. After incubating for 30 minutes at room temperature (i.e. at about 20 °C), 0.025mL of 1% (v/v) chicken Red Blood Cells (RBC) was added to each well, mixed gently and set for about 40 minutes to allow the RBCs to settle at room temperature. Agglutination was assessed by tilting the plates. Each plate also included baseline samples collected from the birds before vaccination (negative controls). The HI titer was the highest dilution of serum causing complete inhibition of the 4 HAU of antigen. HI titers may be regarded as being positive if there was inhibition at a serum dilution rate of 1/16 ( $2^4$  or  $\log_2 4$  when expressed as the reciprocal), or

more against 4 HAU antigen. The serology values stemming from this assay were thus related to the immune status of the birds.

### 3.2.4 Statistical analysis

To investigate the effect of standard husbandry practice on chick weights at 6 weeks and 12 weeks of age, a Generalized Linear Mixed Model (GLMM) was used, with chick weight as the dependent variable. Husbandry practice, year, sex, breed and the interactions between them were entered as fixed factors while sire and dam identity (and their interaction) were entered as random factors, with day-old weight as a linear covariate. The effect of husbandry practices on survival at 6- and 12-weeks-old was investigated using a similar GLMM, with survival as the dependent variable. Husbandry practice, year, sex, breed (and the interactions between these) were entered as fixed factors, while sire and dam identity (and their interaction) as random factors. The binomial data (dead: 0; alive: 1) was linked to the normal distribution using the probit link function. Finally, to investigate the effect of husbandry practices on the immune response at 20 weeks of age, two models were used. Firstly, a GLMM was performed using dilution rate needed to achieve complete inhibition of the antigens as the dependent variable. The fixed factors were husbandry practice, year, sex, breed (and their interactions), while the sire and dam's identification (and their interaction) were entered as random factors. Secondly, a GLMM similar to the one used for survival analysis was performed by linking the binomial data (negative result to the HI test: 0; positive result to the HI test: 1) to the normal distribution using the probit link function. The significance of effects (fixed and covariate) in the GLMMs were examined using Wald Type adjusted F-statistics. The effects with the least significant P-value was sequentially dropped until only significant terms ( $P < 0.05$ ) remained in the model (Jones and Taylor, 1999). All statistical analyses were performed using Genstat version 13 (VSN International Ltd., UK; Jones and Taylor, 1999).

### 3.3 Results

The mean weight of chicks at 6 weeks and 12 weeks of age was  $6.92 \pm 0.27\text{kg}$  and  $22.53 \pm 3.03\text{kg}$ , respectively. No difference was recorded between males and females, or between the different breeds at both ages ( $P > 0.05$ ). The survival rate across all groups was  $86.03 \pm 1.20\%$  at 6 weeks and  $83.30 \pm 1.50\%$  at 12 weeks of age. While no HI titer was detected before the vaccine injection,  $55.60 \pm 1.50\%$  of chicks subsequently showed a positive result to the HI test, and an average ratio of 1/23 was needed to cause complete inhibition of the antigens.

Chicks exposed to extensive human presence (I1 and I2) were heavier than chicks exposed to the S husbandry practice at 6 weeks of age, and even more so for the I1 chicks which received physical stimulation ( $F_{2, 359} = 25.03$ ,  $P = 0.001$ ; Table 3.1). No effect of sex or breed was detected ( $P > 0.05$ ), but a difference was observed between the two years with chicks from the 2013 batch being 22% heavier than chicks from the 2015 batch (2013:  $7.60 \pm 0.14\text{kg}$ ; 2015:  $6.23 \pm 0.09\text{kg}$ ;  $F_{1, 113} = 67.15$ ,  $P = 0.001$ ). At 12 weeks of age, no difference in weight gain was observed between husbandry practices ( $P > 0.05$ ; Table 3.1), or between sex and breed groups ( $P > 0.05$ ; Table 3.1). A weight difference between the two years was also detected but with an opposite trend (as compared to 6 weeks old), whereby chicks from the 2015 batch were 69% heavier than the 2013 batch (2013:  $16.82 \pm 0.28\text{kg}$ ; 2015:  $28.46 \pm 0.35\text{kg}$ ;  $F_{1, 255} = 48.79$ ,  $P = 0.001$ ). Survival analysis showed I1 and I2 chicks survived better than S chicks to 6 weeks of age ( $F_{2, 359} = 4.59$ ,  $P = 0.001$ ; Table 3.1). No effects of sex, breed or year were detected on survival to 6 weeks of age ( $P > 0.05$ ). At 12 weeks of age, survival was independent of husbandry practice, sex, breed and year ( $P > 0.05$ ; Table 3.1).

Table 3.1 The effect of different husbandry practices on ostrich chick survival and live weight at 6 and 12 weeks of age.

Trait	I1	I2	S	F value	DF	P-value
Live weight (kg)						
6 weeks	7.47±0.18 <sup>a</sup>	7.06±0.15 <sup>b</sup>	6.21±0.13 <sup>c</sup>	25.03	2,359	<0.05
12 weeks	22.67±0.63	22.80±0.67	22.10±0.66	0.56	2,354	>0.05
Survival (%)						
0 - 6 weeks	87.5±1.2 <sup>a</sup>	86.9±1.2 <sup>a</sup>	83.7±1.3 <sup>b</sup>	4.59	2,359	<0.05
0 - 12 weeks	83.2±1.1	85.8±1.0	80.9±1.2	0.34	2,354	>0.05

<sup>a b c</sup> means with different superscript within a row differ significantly (P<0.05)

Chicks in the I1 husbandry practice (N=136) were familiarized with human voice, touch and general human presence. Chicks exposed to the I2 husbandry practice (N=144) were also familiarized to human voice and general human presence, but not to physical touch. Chicks exposed to the S husbandry practice (N=136) had human exposure limited to the provision of food and fresh water.

Finally, the immune status of chicks was improved in the I1 group relative to the other husbandry groups. Fewer I1 chicks showed positive results to the HI test compared to I2 and S chicks 21 days post-injection ( $F_{2,292}=3.14$ ,  $P=0.007$ ; Figure 3.1). This was also confirmed by analysing the dilution rate needed to cause complete inhibition of antigens, as I1 chicks required a much lower dilution rate compared to I2 and S chicks (I1: 1/18; I2: 1/25; S: 1/23;  $F_{2,276}=5.04$ ,  $P=0.01$ ; Figure 3.2), suggesting a stronger immune competence in I1 chicks.

Interestingly, a significant effect of breed on immune status was also detected ( $F_{4, 147}=4.49$ ,  $P=0.01$ ) with purebred KR chicks requiring a much higher dilution rate than any of the

other breeds to cause complete inhibition of antigens (Figure 3.3). A significant interaction was also observed between husbandry practices and sex ( $F_{2, 289}=6.87$ ,  $P=0.01$ ). There was no difference between males and females in the I1 and I2 groups. In contrast, males in the S group required a higher dilution rate than females ( $F_{1, 111}=6.13$ ,  $P=0.002$ ).

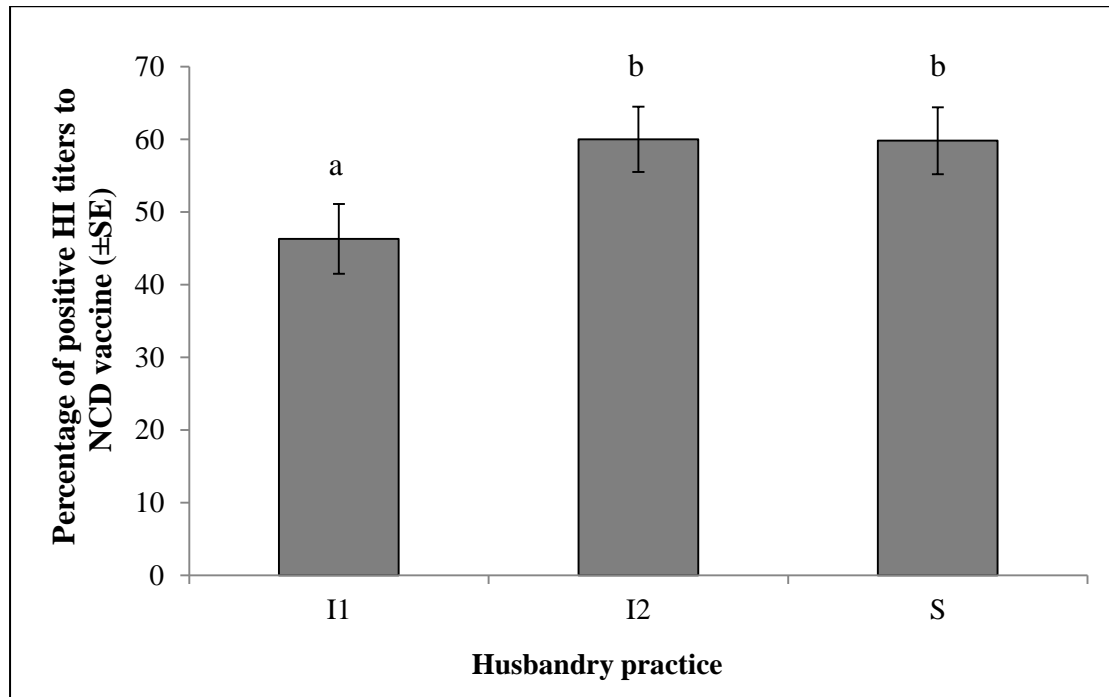


Figure 3.1 Least squares means ( $\pm$ SE) depicting the effects of different husbandry practices on the immune response of 5-month-old ostrich chicks using a Hemagglutinin- Inhibition (HI) test following injection with Newcastle Disease Vaccine for the percentage of positive HI titers. Means with different superscripts differed significantly ( $P<0.05$ ). Husbandry practices: I1 (N=108), I2 (N=120) and S (N=112).

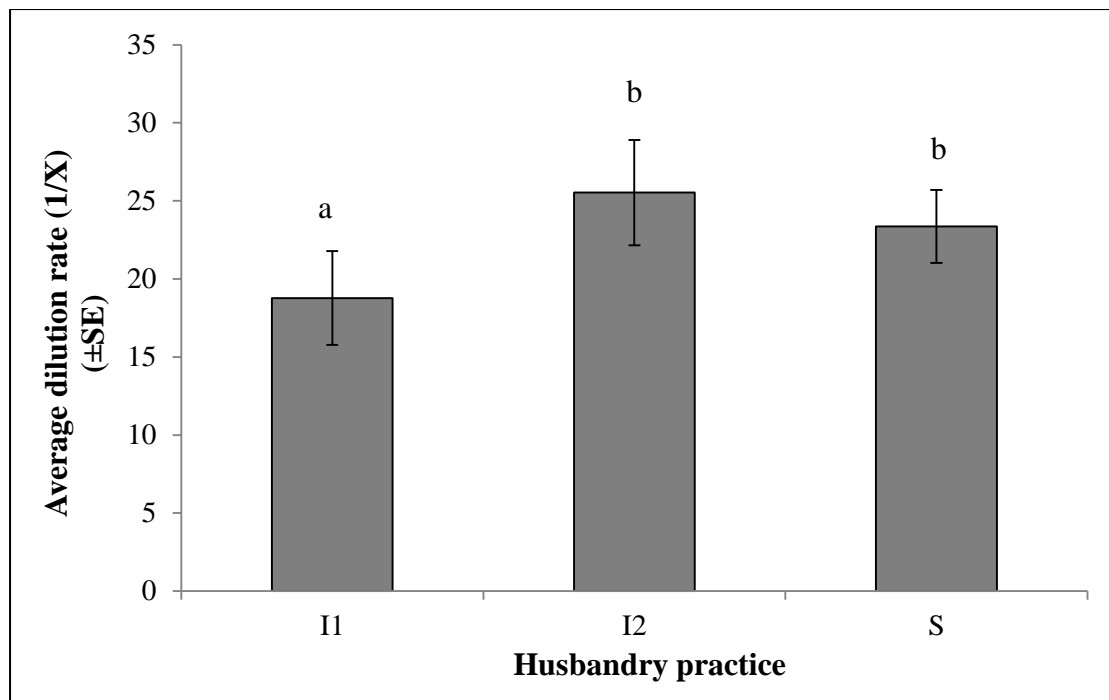


Figure 3.2 Least squares means ( $\pm$ SE) depicting the effects of different husbandry practices on the immune response of 5-month-old ostrich chicks using a Hemagglutinin- Inhibition (HI) test following injection with Newcastle Disease Vaccine for the average dilution rate needed to achieve complete inhibition of the antigens. Means with different superscripts differed significantly ( $P < 0.05$ ). Husbandry practices: I1 (N=108), I2 (N=120) and S (N=112).



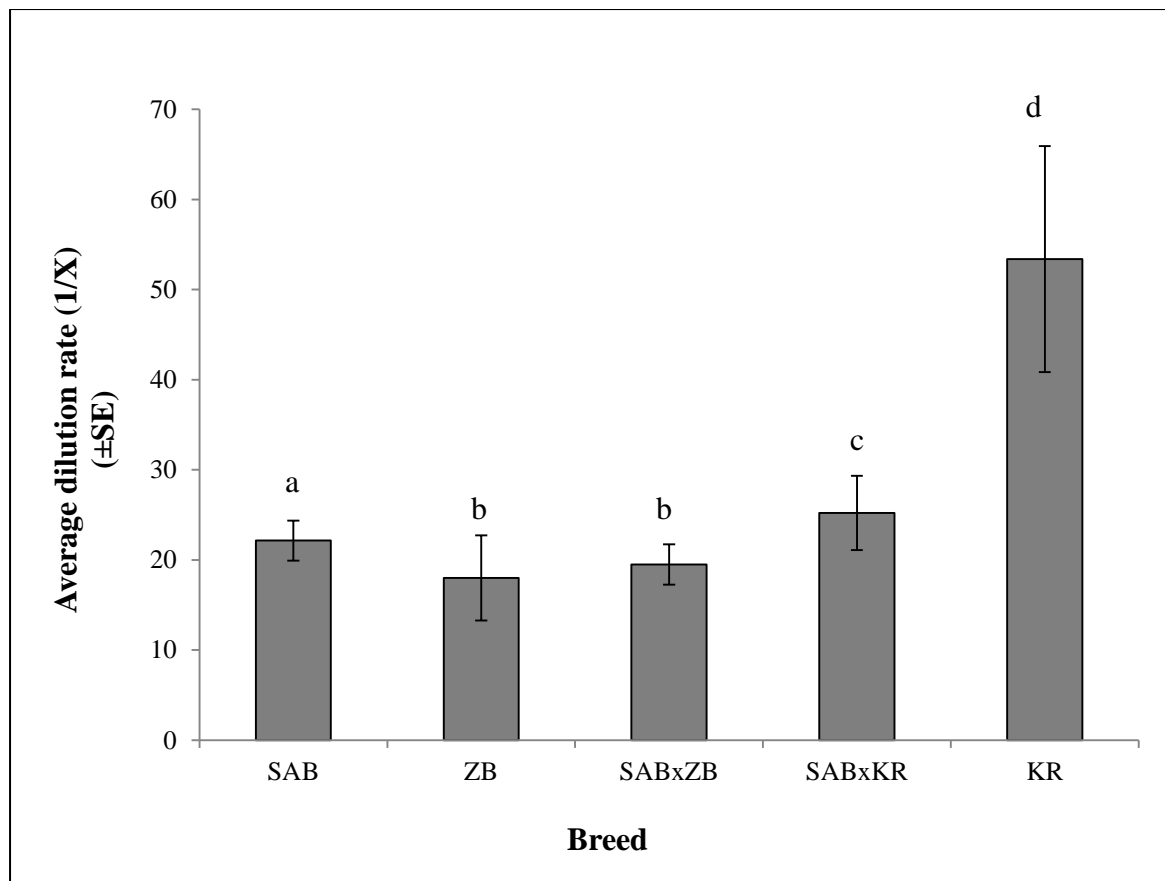


Figure 3.3 Least squares means ( $\pm$ SE) depicting the effects of different husbandry practices on the immune response of 5-month-old ostrich chicks using a Hemagglutinin- Inhibition (HI) test following injection with Newcastle Disease Vaccine for the effect of breed. Means with different superscripts differed significantly ( $P < 0.05$ ). [The breeds were SAB: South African Black (N=204); ZB: Zimbabwean Blue (N=8); KR: Kenyan Redneck (N=8) and their cross-bred combinations: SAB $\times$ ZB (N=71); SAB $\times$ KR (N=49)].

### 3.4 Discussion

The results of this study show that ostrich chicks exposed to extensive human presence achieved heavier body weights at 6 weeks of age than chicks exposed to limited human presence, even more so when chicks experienced human presence with physical interactions (stroking/touch stimuli). Compensatory growth in the standard husbandry group resulted in this advantage being largely eliminated by 12 weeks of age.

Improved early growth rate in ostrich chicks is integral to the production of quality slaughter birds (Verwoerd et al., 1999). The current study revealed that early body weight gain up to six weeks of age may be facilitated by extensive human presence and regular gentle handling in ostrich chicks. However, extensive human care did not significantly improve the growth of ostrich chicks relative to the standard husbandry group to 4 and 9 weeks of age, respectively, in an earlier study (Wang et al., 2012). It needs to be noted that the intensity and duration of extensive human presence in the latter study was less than that of the present study. Previous studies on other species also demonstrated that regular gentle handling and hand feeding of chickens once or twice each day up to 8 weeks old improved weight gain, compared to chickens that were ignored except for essential husbandry needs (Gross and Siegel, 1982; Collins and Siegel, 1987). Moreover, regular gentle handling (picking up and stroking) of broiler chicks in their home pen for 30 seconds every day for 21 days improved weight gain to 46 days of age, compared to chicks that were handled irregularly (Zulkifli and Siti Nor Azah, 2004). However, this method of human-animal interactions may have an impact on the weight gain during the growth period. Weight gain was not affected when broiler chicks were exposed to human presence only, without physical and audio interactions with the chicks (Zulkifli et al., 2002). Furthermore, Leonard and Fairfull (1992) could not find any significant difference in weight gain between White Leghorn chickens that were handled gently at an early age and contemporaries not handled. In their study, handling was done by gently removing the chicks from their home cage to a large cardboard box. Moving the chicks into a novel confinement might have induced stress in the latter study, which could have affected their growth since the chicks were not familiar with the cardboard box.

Experimental ostrich chicks from the 2013 batch were heavier than the 2015 batch after 6 weeks, while the opposite was true at 12 weeks of age. The time of the year has been

described previously as having an effect on the growth rate of ostrich chicks, with chicks hatched in spring growing faster than chicks hatched in other seasons (Verwoerd et al., 1999). All chicks in this study (I1, I2 and S) were reared in an intensive chick rearing facility and were taken outside regularly when the weather was permitting. At night the ostrich chicks were taken back to the building equipped with artificial heaters (Bunter, 2002). Thus, climatic changes or day light temperature variations may also have resulted in the recorded differences between years. In general, growth in animals during early life is important and any circumstances that may compromise growth at this stage is likely to have a detrimental effect on survival or the full expression of an animal's genetic potential (Cloete et al., 2001; Deeming, 2011).

Survival of ostrich chicks in commercial farming conditions is highly variable, especially during the period between hatching and three months of age before chicks gain independence (Verwoerd et al., 1999; Cloete et al., 2001). A higher rate of chick mortality up to 78.4% before the age of three months have been recorded in South Africa over two successive breeding seasons (Cloete et al., 2001). However, most chicks died before four weeks of age, while daily mortality rates declined considerably thereafter, indicating that as the chicks gained more weight the chances of dying were reduced (Cloete et al., 2001). This is in accordance with the results of Verwoerd et al. (1999) who revealed reduced mortalities between 3 and 12 months of age. This emphasises the importance of maintaining high early survival rates since it is likely that chicks that survive this period are less likely to succumb before slaughter age. During this study it was apparent that higher survival rates to 6 weeks of age were attainable with exposure to extensive human presence, compared to the standard husbandry group with limited human presence. These findings are comparable with that of Wang et al. (2012) who reported survival rates of 97% to 4 weeks of age in ostrich chicks exposed to extensive human presence and care, compared to 84% in chicks reared by

standard husbandry practices. In contrast, extensive human presence only (without physical and audio interactions) did not affect mortality in broiler chicks (Zulkifli et al., 2002). In the current study, survival to 12 weeks of age was independent of husbandry practice, while sex, breed and year also did not affect survival to 6 or 12 weeks of age.

The immune system is the first line of defence against disease causing micro-organisms, and accordingly coordinates a protective immune response in birds (Girard et al., 2011). Thus, any physiological condition that challenges the immune system may compromise the immune response. In chickens, Gross and Siegel (1982) reported an improved antibody response to an antigen in chickens that were handled gently at an early age, compared to chickens of the same age that were ignored during rearing. Another study that comprised of different human activities with the chicks demonstrated an improved antibody response for a group of chickens that received extensive visual human contact compared to chickens that had limited visual human contact (Zulkifli et al., 2002). The results of the current study are in agreement with these findings, as it was found that the immune status of ostrich chicks was improved in the I1 group compared to I2 and S groups 21 days post Newcastle vaccine injection.

The dilution rate needed to cause a complete inhibition of antigens was lower for I1 chicks compared to I2 and S chicks. This suggests that I1 chicks had a notably stronger immune response as discussed previously. Furthermore, a significant effect of breed was detected, with purebred KR chicks requiring a much higher dilution rate than any other breeds or breed combinations. As the KR ostrich breed was more recently domesticated, compared to the SAB and ZB strains (Bunter, 2002), KR chicks may not yet have adapted to commercial farming conditions and consequently experienced higher levels of stress. However, further studies are needed to clarify the main causes of these differences between breeds.

A significant interaction was also observed between husbandry practice and sex. While no difference was recorded in the dilution rate required to cause a complete inhibition of an antigen between males and females in the I1 and I2 groups, males in the S group required a higher dilution rate than females. Hence male ostrich chicks reared using the S husbandry practice may have experienced severe stress which resulted in increased dilution rate required to cause a complete inhibition of an antigen. In contrast, rearing chicks using extensive human presence and care appeared to reduce stress in both sexes. Consequently, factors leading to such differences between male and female ostrich chicks subjected to the S husbandry practice may require further studies.

### 3.5 Conclusions

Ostrich chicks subjected to extensive human presence along with physical stimuli early in life seemed to benefit on various levels. These chicks achieved a heavier 6-week-old body weight than chicks reared according to the standard rearing method commonly used on commercial ostrich farms. In addition, their survival to 6 weeks and immune competence at 20 weeks of age was improved. These results indicate that chicks exposed to extensive human presence were able to better tolerate early life stressors, thus enabling them to bridge the most critical phase prior to gaining independence. Thus, integrating extensive human presence along with positive human-chick interactions in currently used husbandry protocols may alleviate common problems related to chick rearing and improve chick welfare. However, commercial ostrich chicks are not always reared in smaller groups such as used in this study. Therefore, there is a need to further investigate the effect of extensive human presence at various stocking densities to establish the maximum number of birds per group that can be subjected to extensive human presence during this stage of life to obtain the benefits shown in this study.

### 3.6 Acknowledgements

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## **Chapter 4**

# **Extensive human presence at an early age reduces short- and long-term stress sensitivity in ostriches, but not docility at a later age**

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**(Appl. Anim. Behav. Sci., under review)**

## Abstract

Positive human-animal interactions have been shown to alleviate problems associated with animal handling (i.e. stress, risk of injuries, etc) and improve animal welfare. Hence, this study investigated the effect of human presence and regular gentle handling, performed at an early age, on stress responses and docility of juvenile ostriches. A total of 416 ostrich chicks were reared under husbandry practices which varied in human presence and human-chick interactions (I1: extensive human presence with audio, visual and touch stimuli; I2: extensive human presence with audio and visual stimuli and S: human presence limited to feed and water supply) up to 3 months of age. Short-term stress responses were measured when birds were 7.5 months old, by quantification of the plasma heterophil/lymphocyte (H/L) ratio, before and 72 hours after feather harvesting and feather clipping. Long-term stress responses were measured by quantification of corticosterone (CORT) concentrations in the floss feathers. Social behaviours towards human such as a bird's willingness to approach, allow touch interactions and exhibit sexual display, were recorded 3 times a week from 8 to 13 months of age. Docility and fear test were performed when the birds were 12 months of age. There was no difference in the H/L ratio of I1 birds before and after feather harvesting and clipping ( $P>0.05$ ), while H/L ratios of both I2 and S birds showed a significant increase 72 hours post feather harvesting and clipping ( $P<0.05$ ). Furthermore, the feathers of the S group of birds contained a significantly higher CORT concentration, compared to I1 birds ( $P<0.05$ ), while CORT concentrations in I2 birds were intermediate between those of either S or I1 birds ( $P>0.05$ ). No difference was recorded in the bird's willingness to approach and allow touch interactions, sexual display, docility and fear test indicators between the three husbandry practices ( $P>0.05$ ). However, I1 and I2 birds were significantly more inclined to associate with a familiar rather than an unfamiliar handler ( $P<0.05$ ). The results indicate that extensive human presence and regular gentle handling of ostrich chicks at an early age not

only reduces short- and long-term stress sensitivity later in the life of ostrich chicks, but also that welfare of these relatively wild animals could be improved when handling is facilitated by a familiar handler.

#### 4.1 Introduction

Research pertaining to commercial ostrich farming began approximately 100 years ago with an exponential increase during the past 15 years (Cloete et al., 2012). Although considerable progress has been made in most areas (Cloete et al., 2008; 2012), commercial ostrich farming does not incorporate selection of stress resistant birds that exhibit docile behaviour as replacement breeders (Amado et al., 2011). Hence, the management of farmed ostriches remains a challenge, as husbandry practices for chicks are not optimised (Deeming, 2011). Furthermore, the relatively wild behaviour exhibited by mature birds compromises not only the welfare of the birds but also the safety of handlers (Lambrechts et al., 2000; Cloete et al., 2012).

There is a growing interest in integrating positive human-animal interactions in the husbandry practices of livestock species (poultry, cattle and pigs), since such interactions have been demonstrated to reduce fear of humans, and to improve animal welfare, docility, and production (Hemsworth, 2003; Waiblinger et al., 2006; Bertenshaw et al., 2008; Zulkifli, 2013 Lürzel et al., 2017; Tallet et al., 2018). For instance, regular gentle handling (i.e. stroking, patting, hand resting on the animal) decreased stress levels in broiler chicks after transportation for 3 hours (as indicated by a lower heterophil/lymphocyte ratio and plasma corticosterone concentration at 7 weeks of age; Al-Aqil et al., 2013). Similarly, dairy cattle (Breuer et al., 2003) and pigs (Hemsworth et al., 1986) exposed to positive human handling had lower corticosteroid concentrations, as compared to animals exposed to negative handling. The lower levels of heterophil/lymphocyte ratio, corticosterone and corticosteroid

concentrations in positively handled animals are a result of lower physiological changes of the stress response mechanism (i.e. the hypothalamic-pituitary-adrenal axis) which indicate less chronic stress (Hemsworth and Coleman, 2011).

The importance of positive human-animal interactions in improving docility has been demonstrated in previous studies. Hemsworth et al. (1994) showed that exposing broiler chicks to regular gentle handling at an early age reduced their withdrawal behavioural responses from an approaching human at later stages of life. Similarly, dairy heifers that received extended human care and regular gentle handling such as brushing were reported to show less fear of humans. They demonstrated improved milking parlour behaviour and delivered less kicks at humans than those receiving limited human presence and care (Bertenshaw et al., 2008). Those positively handled dairy heifers also kept a greater distance when approached by an unfamiliar compared to a familiar handler, suggesting that they could distinguish between handlers and adjust their behaviour accordingly (Breuer et al., 2003). Preliminary work by Bonato et al. (2013) demonstrated that ostrich chicks exposed to husbandry practices that incorporated extensive human interactions at an early age were more inclined to associate with humans than those reared under husbandry practices with limited or no human presence. Thus, the aim of this study was to investigate the effect of three different husbandry practices with varying levels of human presence and interaction on short- and long-term physiological stress responses, social behavioural responses, docility and fear responses of juvenile ostriches towards human.

## 4.2 Materials and methods

### 4.2.1 Sampling population and study area

The study site, sampling population, as well as the management of the flock from which the chicks originated has been described earlier (Chapter 3; Bunter and Cloete, 2004; Cloete et

al., 2008). The chicks used in this study (N=416) originated from three purebred ostrich breeds, South African Blacks (SAB), Zimbabwean Blues (ZB), Kenyan Reds (KR) and the reciprocal crossbred combinations of SAB with ZB and KR, respectively. Chicks were exposed to three different husbandry practices, from day-old to 3-months of age, over a period of 2 years, as described in Chapter 3. Briefly, the chicks were provided with extensive human presence with visual, audio and touch stimuli (I1), extensive human presence with visual and audio stimuli only (I2), and human presence limited to the supply of food and fresh water according to the standard husbandry practice (S) used at the Oudtshoorn Research Farm (Bunter, 2002). The duration of exposure to humans was decreased on a weekly basis by 50% (100% of daylight hours during the first week after hatching) until the chicks were 3 months old when the extensive human presence phase was terminated and chicks mixed as one group. Ethical clearance to conduct this study was granted by the Western Cape Department of Agriculture's Departmental Ethical Committee for Research on Animals (Ref No.: R13/81.).

#### 4.2.2 Measurements of stress responses

Short- and long-term stress responses were measured at 7.5 months of age. Short term stress was assessed as birds underwent feather harvesting (2013 and 2015 groups; N=238) and feather clipping (2015 group only; N=87). During feather harvesting, birds were caught and restrained and ripe feathers were gently pulled, while feather clipping involved the cutting of feathers above the base of the shaft (Shanawany and Dingle, 1999). A drop of blood was also collected from the wing vein before and 72 hours after each feather treatment (N=325) for basal heterophil/lymphocyte (H/L) ratio and peak stress responses respectively (Romero and Romero, 2002). The blood smears were air-dried and fixed by immersion in 99.9% methanol, in a coplin glass jar, for 3 minutes on the day of collection. After fixation blood smears were stained with 10% Giemsa solution for 45 minutes, rinsed, air dried and stored until analysis.

The H/L ratio was assessed from a blood smear by counting one hundred white blood cells per slide.

To assess long-term stress caused by routine farm management practices (i.e. weighing, tagging, health inspection, general human presence and movement of chicks) the corticosterone (CORT) concentrations was quantified in the floss feathers collected during the feather harvesting. The procedure for quantifying CORT concentration levels in the feathers was adapted from Bortolotti et al. (2008). The feathers were harvested from 48 SAB ostriches (2015 group only; 16 from each husbandry practice; 8 males and 8 females) and stored in sealed bags at room temperature until analysed. After removal of the calamus, the length of the feathers was measured before cutting into three equal sections (bottom, middle and top section). Each section was weighed, and the rachis removed. Only the top (growth during the treatment period) and bottom sections were used for analyses. To remove any external contaminants, the sections were washed in a sieve with water and ethanol for 20 and 10 seconds, respectively. Scissors were then used to cut both vanes into fine pieces of less than 5mm<sup>2</sup>. Cut sections were allowed to air dry before 20mg was placed in labelled 20ml borosilicate vials. A volume of 10ml methanol was added to each vial before they were sonicated for 20 minutes and placed in a shaking incubator overnight for 17 hours at 52 °C at 100 rotations per minute. Subsequently, a volume of 8ml methanol was retained from each vial prior to the samples being washed twice with 2.5ml of methanol, 2ml being recovered each time. The pooled extracts (12ml) were filtered through 10ml plastic syringes attached to 0.45µm Ministart® high flow syringe filters (Sartorius AG, Göttingen, Germany) and collected in 12.5ml borosilicate test tubes. All methanol samples were subsequently evaporated using a sample concentrator at 48 °C in a standard airflow fume hood. Care was taken to avoid over drying, before samples were reconstituted in 240µl of enzyme-linked immunosorbent assay (ELISA) buffer and vortexed for 15 minutes to ensure samples were

thoroughly mixed. All samples were assayed in duplicate, using a commercial Cayman CORT ELISA assay according to the manufacturer instructions (Item no. 501320, Cayman Chemical, c2016). Optical density of samples was measured with the aid of spectrophotometer. The concentration of CORT in each sample was then calculated using online software from ElisaAnalysis.com (Elisakit.com, Pty Ltd, c2012) using a 4-parameter logistic fit. Concentrations were finally normalised according to section sample weight (pg/mg).

#### 4.2.3 Social behavioural observations, docility and fear test

##### 4.2.3.1 Social behavioural test

Social behavioural observations were conducted 3 times a week from 8 months to 13 months of age (N=207) by two handlers (one familiar and one unfamiliar to the birds), that wore similar clothing. The familiar handler interacted with the birds during the first 3 months they were exposed to the I1 and I2 husbandry practices while the unfamiliar handler did not. The procedure of observing and recording the behavioural responses towards the handler has been described previously by Bonato et al. (2013). Briefly, during the observation sessions 20 birds (10 males and 10 females) were randomly selected and their specific behaviours towards the handlers recorded. Behaviours such as approach (bird coming towards the handler), touch (bird could voluntarily allow to be touched by the handler) and wing flapping (bird raising feathers up and down as the human approached) were recorded. In addition, sexual displays towards the handler, such as kantling, stepping and clucking by males as well as crouching and clucking by females were recorded (Malecki and Rybnik-Trzaskowska, 2011; Bonato et al., 2015). Negative behaviours such as avoidance (maintaining distance from the handler), excessive pecking (repeatedly grabbing the handler's body or clothes) and aggression (hissing and/or kicking at the handler) were also recorded. The expression or lack



of expression of these behavioural traits was recorded in a binomial format as 1 or 0, respectively. The behavioural observations were performed when all birds were maintained together in groups of mixed sex within hatching years and not separated according to husbandry practices.

#### 4.2.3.2 Docility test

The docility test, performed in 2013 (N=32) and 2015 (N=91), was adapted from descriptions by Mazurek et al. (2011). In each year, a small group of birds (between 15 and 20) was randomly allocated from the main flock and moved to a holding pen closer to the test arena. The test arena (29m×35m) was composed of a square (8m×8m) drawn on the floor at a corner opposite to the holding pen containing the peers (Figure 4.1). One bird at a time was randomly chosen from the holding pen and guided gently to the test arena by two experienced stockmen. Each bird was released at the gate of the test arena and given 10 seconds to familiarize itself with the test pen before the onset of the test. To investigate whether the birds could discriminate between familiar and unfamiliar individuals, each bird was tested by a familiar and unfamiliar handler. When birds were brought to the test arena the handler was standing approximately 3m away from the test arena gate. The handler attempted to encourage the bird to enter a marked square in the corner of the test arena for 30 seconds using slow arm movements and a calm voice (Figure 4.1). The test was terminated after the bird was contained in the box for 30 seconds, or if the bird was not moved into the marked square within 3 minutes, or when it threatened or charged the handler. The test was considered successful if the bird could be contained in the marked square for 30 seconds. The time taken by the bird to enter the marked square, as well as the time the bird was contained in the marked square was recorded to evaluate docility. Birds that threatened/charged the handler were recorded as being aggressive. Defecation and frequent vocalisation were also

recorded since they may indicate stressful responses during handling (Hemsworth et al., 2011; Bejaei and Cheng, 2014).

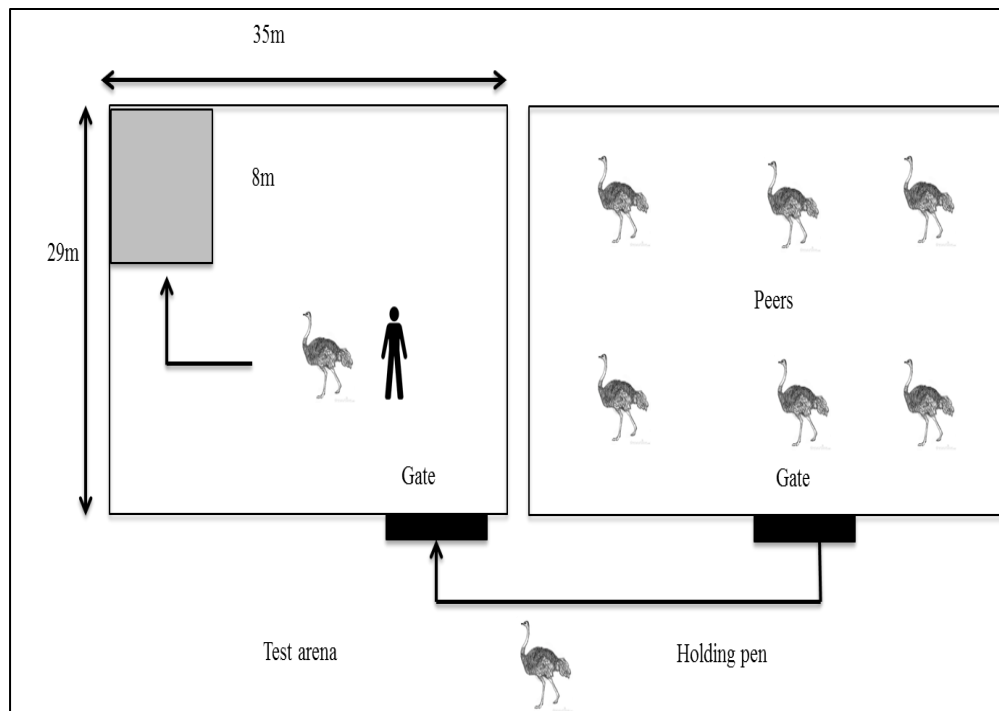


Figure 4.1 Experimental procedure used during the docility test showing the holding pen (with peers) and the test arena (with a marked square drawn on the corner opposite the gate).

#### 4.2.3.3 Fear test

Similarly to the docility test, the fear test was adapted from Mazurek et al. (2011). The test arena was designed by dividing a pen (55m×29m) into 18 equal squares drawn on the floor (9.4m×9.4m; Figure 4.2). These drawn squares were assigned a different score value according to their distance from the experimenter. The scores were assigned as follows: 0 for the square containing the feed trough, 1 for the adjacent squares, then 2, 3, 4 and 5 for the squares progressively further away (Figure 4.2). A small group of birds (15-20 birds) was randomly allocated from the main flock (2013 only: N=83) and moved into a holding pen adjacent to the test arena. In the holding pen, the birds were supplied with food and fresh water. One bird at a time was randomly chosen from the group in the holding pen and gently

guided to the test arena by an experienced stockperson, until all birds were tested. The bird was released at the gate of the test arena, while the handler was standing approximately 3m away from the gate, and given 10 seconds to familiarize itself with the arena before the test was started. Overall, the test was conducted for 4 minutes and was composed of three phases. Phase 1; the bird was left alone in the arena (1 minute); Phase 2; the handler entered the arena, placed food in the feed trough and then walked out (1 minute); Phase 3; the handler entered the test arena and offered food to the bird (2 minutes). Across all phases the bird could see its peers in the holding pen. The number of squares crossed in each phase was recorded, as well as the position of the bird to calculate the distance the bird kept from the stimulus square. A square area was considered crossed if the bird placed both feet in it. During the second and third phases, the time taken for the bird to feed after the handler put food in the feed trough and while the experimenter was standing close to the feed trough was recorded. Furthermore, the time taken for each bird to interact with the experimenter (interacting either by pecking or feeding from the experimenter's hand) was recorded in Phase 3. Each interaction was further recorded in a binomial format (1: interacted with the handler and 0: did not interact with the handler). The fear test was conducted by both familiar and unfamiliar handlers.

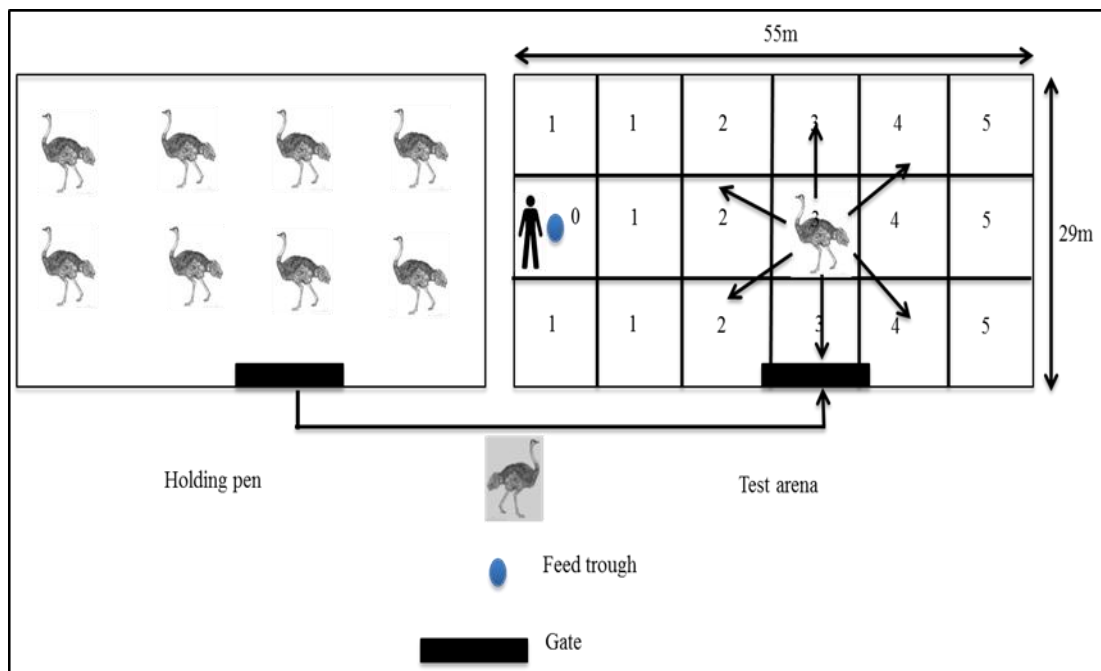


Figure 4.2 Experimental procedure of the fear test showing the holding pen (with the peers) and the test arena (divided into 18 equal squares).

#### 4.2.4 Statistical analysis

Due to staffing limitations some of tests were only conducted in one experimental year. Therefore, the year corresponding to each test was indicated as well as the number of birds used for that specific test. To compare the overall H/L ratio before and 72 hours after feather harvesting and feather clipping, a paired t-test was performed for each husbandry practice. A Generalized Linear Mixed Model (GLMM) was used to evaluate the effect of the husbandry practice on short-term stress responses where the H/L ratio was entered as dependent variable. The breed, sex, husbandry practice, method of feather removal (feather harvesting and feather clipping), year and their interactions was entered as fixed factors. Bird identity was included in the model as a random variable to account for pre- and post-sampling. The effect of husbandry practices on long-term stress responses was evaluated using a similar GLMM, with CORT concentrations as dependent variable, while husbandry practice, sex, feather section and their interactions were entered as fixed factors. The weight of the birds

was entered as a linear covariate, with bird identity as a random variable to account for repeated records on the same individual. The CORT concentration data were log transformed to follow a normal distribution assumption. The data on social behavioural responses were recorded in binomial format (0 or 1) and normalized using the logit link function of the GLMM. To evaluate the effect of husbandry practices on social behavioural responses, these were entered as dependent variables. Husbandry practice, year, familiarity, sex, breed and interactions between sex and husbandry practice, as well as year and husbandry practice were entered as fixed factors. The bird identity was entered as a random variable to account for repeated records on the same individual. All social behavioural responses, except for approach/avoiding the experimenter were analysed based on data of birds that approached the observer. The time taken by birds to enter the marked square drawn on the ground and the period of time the bird was contained in the marked square during the docility test were entered as dependent variables in a GLMM. The husbandry practices, year, familiarity of the handler, sex, breed and the interaction between sex and husbandry practices, as well as year and husbandry practices were entered as fixed factors. Bird identity was entered as a random variable to account for repeated records on the same individual. A similar GLMM was performed to evaluate the rate of successfully containing the birds in the marked square for 30 seconds during the docility test.

A GLMM was performed for the fear test using the number of squares crossed during each phase, the distance each bird kept away from the stimulus square, as well as time taken to feed or interact with the handler, as dependent variables. The dependent variables were log transformed. Husbandry practices, sex, breed and the interaction between husbandry practices and sex were entered as fixed factors. Bird identity was entered as random variable to account for repeated records on the same individual. Furthermore, a similar GLMM was performed to analyse whether or not the bird interacted with the handler, recorded in a binomial format and

normalized using the logit link function. The PDIFF pairwise comparison was used for the long-term stress responses since the number of observations was balanced across husbandry practices (Kendal, 1993), while the Tukey pairwise comparison was used for the short-term stress responses, social behavioural responses, docility and fear test due to unbalanced number of observations. Statistical significance was set to  $P < 0.05$  and all analyses were performed using SAS, version 9.3 (SAS, 2012).

## 4.3 Results

### 4.3.1 Short- and long-term stress responses

Overall, the plasma heterophil/lymphocyte ratio (H/L) was significantly higher 72 hours after compared to before feather harvesting ( $t = -3.18$ ,  $df = 319$ ,  $P = 0.002$ ; Figure 4.3) and feather clipping ( $t = -5.41$ ,  $df = 121$ ,  $P = 0.001$ ; Figure 4.3). Furthermore, the H/L ratio after feather harvesting was significantly higher, compared to when feather clipping was used ( $t = -4.61$ ,  $df = 298$ ,  $P = 0.001$ ; Figure 4.3).

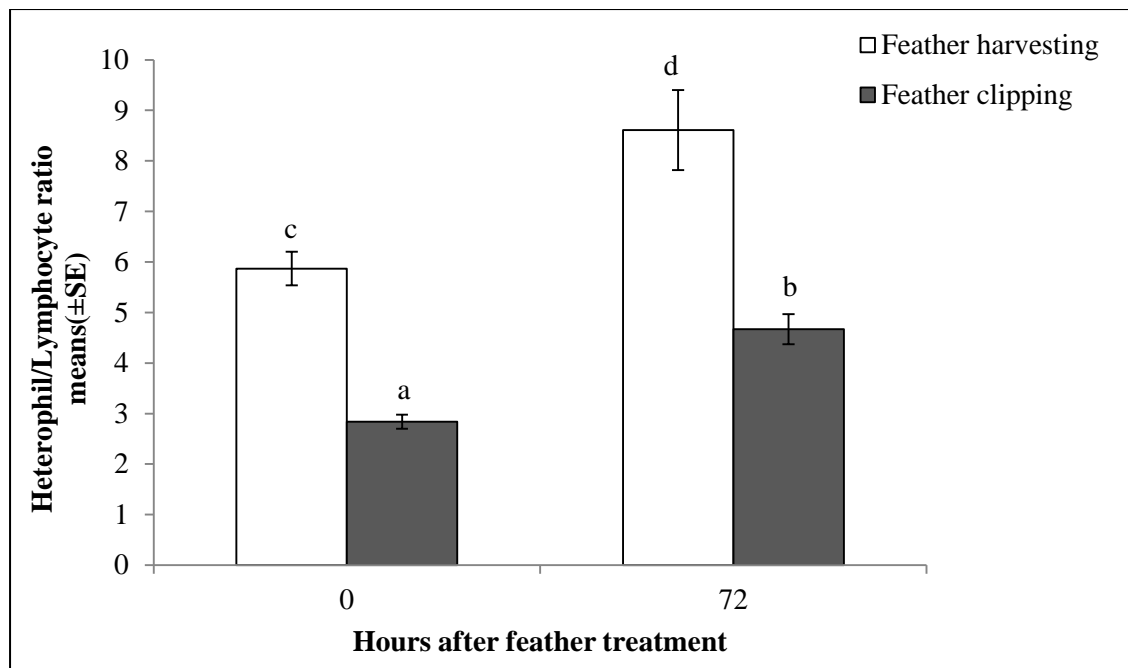


Figure 4.3 The mean plasma heterophil/lymphocyte ratio (H/L) before and 72 hours after feather harvesting (N=238) and feather clipping (N=87) as a measure of short-term stress response of 7.5 months old ostriches. Means with different superscripts differed significantly ( $P < 0.05$ ).

Feather harvesting and feather clipping resulted in a significantly ( $P < 0.05$ ) higher mean H/L ratio 72 hours after compared to before the manipulation in I2 and S birds, but this difference was not seen in the I1 birds (Figure 4.4 and 4.5). There was no effect of breed, sex or interaction between breed and sex on the H/L ratio before and after the feather manipulations. A significant year effect was however recorded, whereby H/L ratio pre- and post-feather harvesting was higher in 2013 compared to 2015 (2013:  $H/L_0$  vs.  $H/L_{72}$ :  $7.61 \pm 0.32$  vs.  $10.84 \pm 0.51$ ; 2015:  $2.75 \pm 0.13$  vs.  $4.57 \pm 0.30$ ;  $P < 0.01$ ). The concentration of CORT in feathers was independent of the sections analysed ( $P > 0.05$ ). Mean CORT concentrations in feathers were significantly higher in the S birds compared to birds from I1 husbandry practice ( $F_{2,42} = 4.23$   $P = 0.02$ ; Figure 4.6), but there was no difference between birds from I1 vs. I2 and I2

vs. S ( $P>0.05$ ; Figure 4.6). There was no significant effect of sex, breed and their interactions for the long-term stress responses ( $P>0.05$ ).

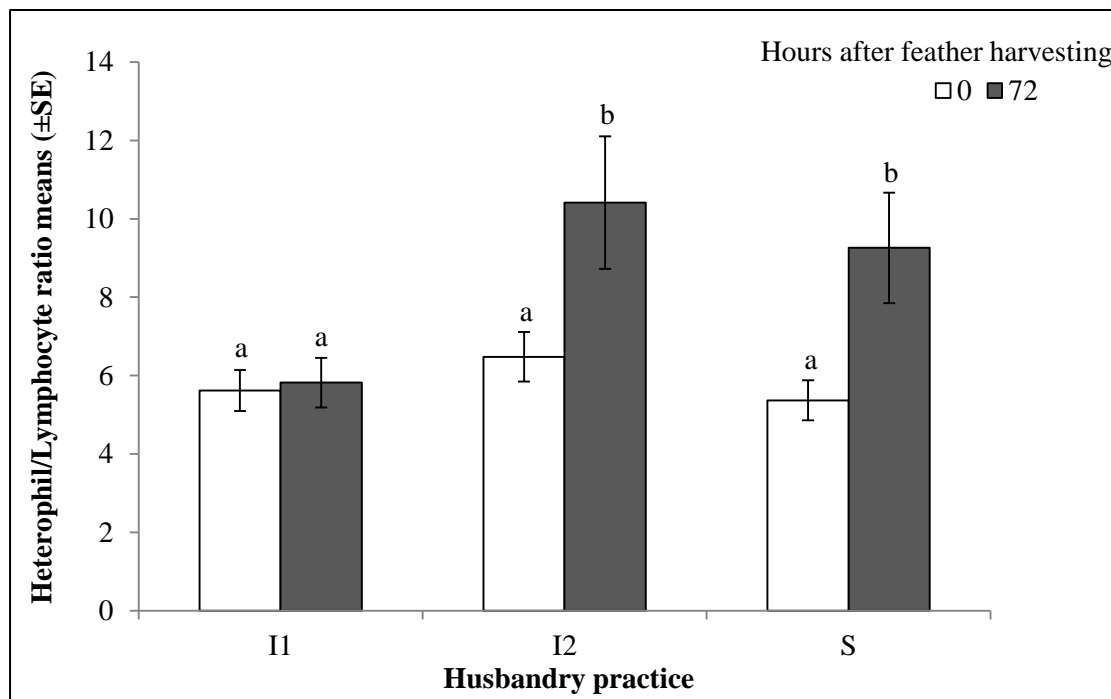


Figure 4.4 The mean heterophil/lymphocyte ratios (H/L) of 7.5-months-old ostriches before and 72 hours after feather harvesting of birds exposed to husbandry practices that differed in the extent of human presence and interactions with the birds. Means with different superscripts differed significantly ( $P<0.05$ ). Husbandry practices: I1 (N=100), I2 (N=119) and S (105).



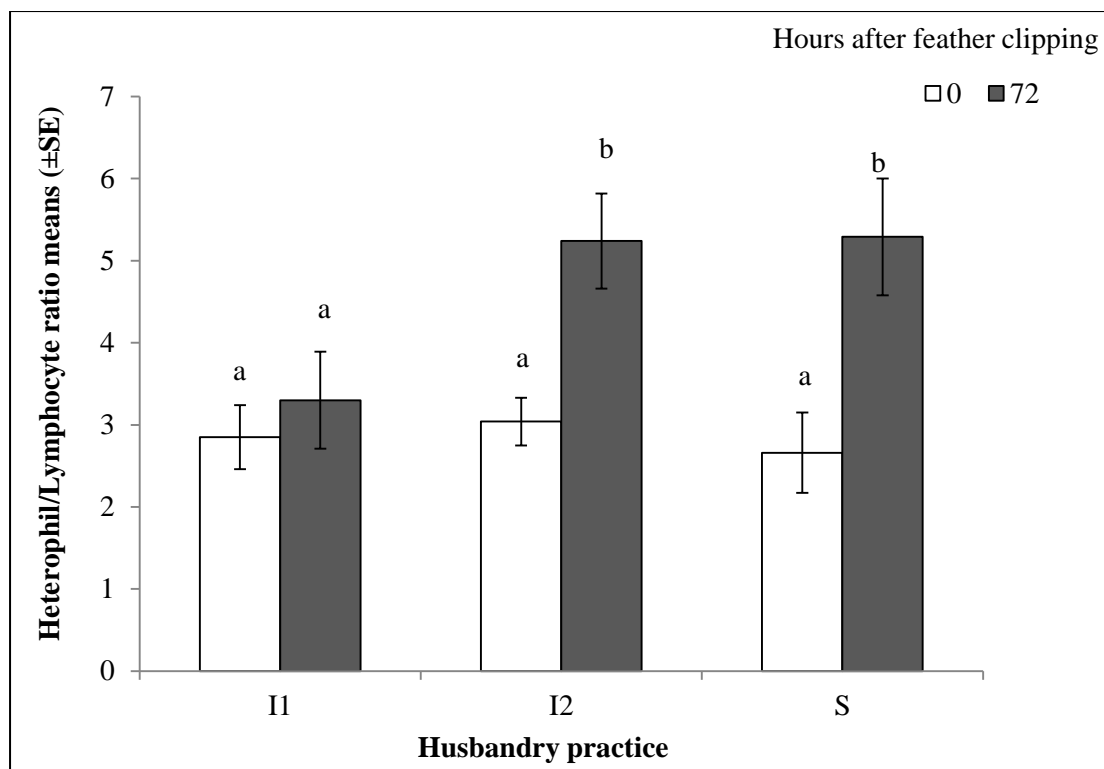


Figure 4.5 The mean plasma heterophil/lymphocyte ratios (H/L) of 7.5-months-old ostriches before and 72 hours after feather clipping of birds exposed to husbandry practices that differed in the extent of human presence and their interactions with the birds. Means with different superscripts differed significantly ( $P < 0.05$ ). Husbandry practices: I1 (N=100), I2 (N=119) and S (N=105).

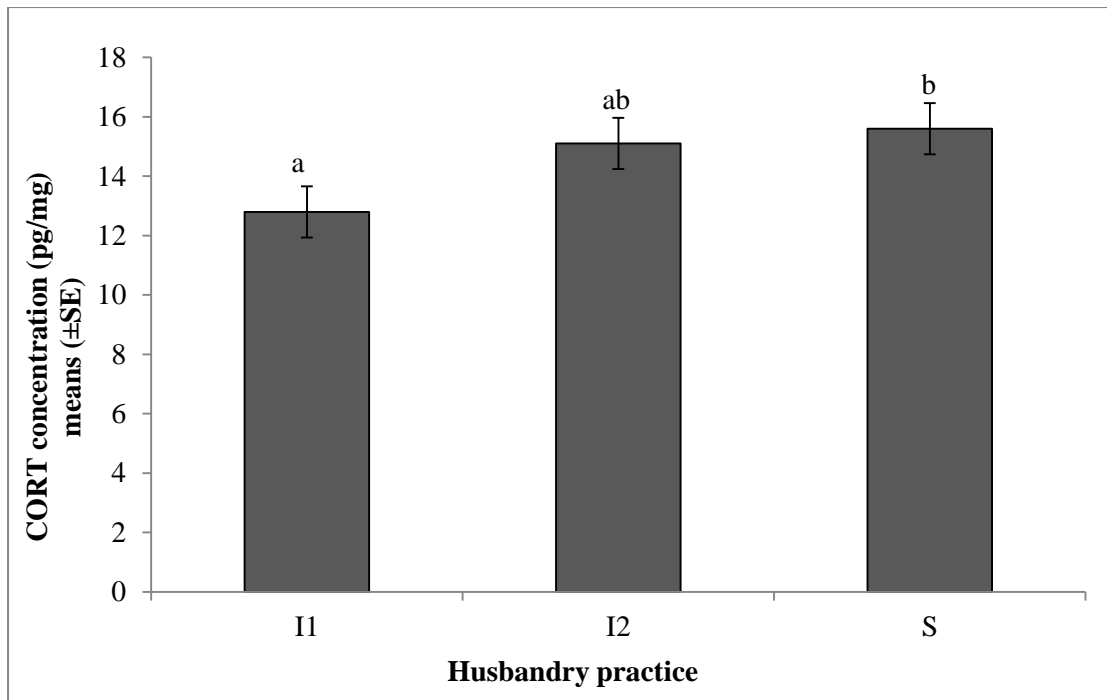


Figure 4.6 The effect of different husbandry practices varying in the degree of human exposure and human-bird interactions on the corticosterone (CORT) concentration of floss feathers of 7.5 months old ostrich chicks. Means with different superscripts differed significantly ( $P < 0.05$ ). Husbandry practices: I1 (N=16), I2 (N=16) and S (N=16).

#### 4.3.2 Social behavioural responses

There was no significant effect of husbandry practices on the social and sexual behavioural responses of birds ( $P > 0.05$ ). I1 and I2 birds were more likely to approach a familiar handler than an unfamiliar handler ( $F_{1, 1168} = 11.3$ ,  $P = 0.008$ ; Table 4.1). Wing flapping was mostly directed at an unfamiliar handler by birds that approached the handlers ( $F_{1, 1112} = 3.91$ ,  $P = 0.04$ ; Table 4.1), but was unaffected by husbandry practices. Birds performing wing flapping differed significantly according to breed, with SAB birds displaying more wing flapping than ZB birds ( $F_{4, 1112} = 5.13$ ,  $P = 0.001$ ; Figure 4.7). Experimental year had a significant effect on whether the birds would allow a human to touch them, with birds from the 2015 group more likely to allow touch by the human than the 2013 group ( $F_{1, 1112} = 5.3$ ,  $P = 0.02$ ; Table 4.1).

There was no significant interaction among fixed effects for any of the other traits measured ( $P>0.05$ ).

Table 4.1 Least square mean estimates ( $\pm$ S.E) of familiarity, experimental year and sex on ostrich social behavioural activities towards the human handler.

Effects	Sexual behavioural response				
	Approach	Touch	Wing flapping	Excessive pecking	Hissing
Familiarity:					
Familiar	3.95 $\pm$ 0.45	2.87 $\pm$ 0.41	0.38 $\pm$ 0.20	2.23 $\pm$ 0.36	4.29 $\pm$ 1.02
Unfamiliar	1.99 $\pm$ 0.36	2.07 $\pm$ 0.37	0.96 $\pm$ 0.21	2.30 $\pm$ 0.37	4.36 $\pm$ 1.05
P-value	<0.01	>0.05	<0.05	>0.05	>0.05
Year:					
2013	3.34 $\pm$ 0.45	2.05 $\pm$ 0.34	0.61 $\pm$ 0.19	2.10 $\pm$ 0.34	3.34 $\pm$ 0.62
2015	2.59 $\pm$ 0.33	2.89 $\pm$ 0.33	0.72 $\pm$ 0.16	2.42 $\pm$ 0.33	5.32 $\pm$ 0.79
P-value	>0.05	<0.05	>0.05	>0.05	<0.05
Sex:					
Males	2.69 $\pm$ 0.30	2.50 $\pm$ 0.31	0.76 $\pm$ 0.17	2.63 $\pm$ 0.33	4.42 $\pm$ 0.60
Females	3.25 $\pm$ 0.37	2.44 $\pm$ 0.31	0.58 $\pm$ 0.17	1.90 $\pm$ 0.32	4.23 $\pm$ 0.61
P-value	<0.05	>0.05	>0.05	<0.01	>0.05

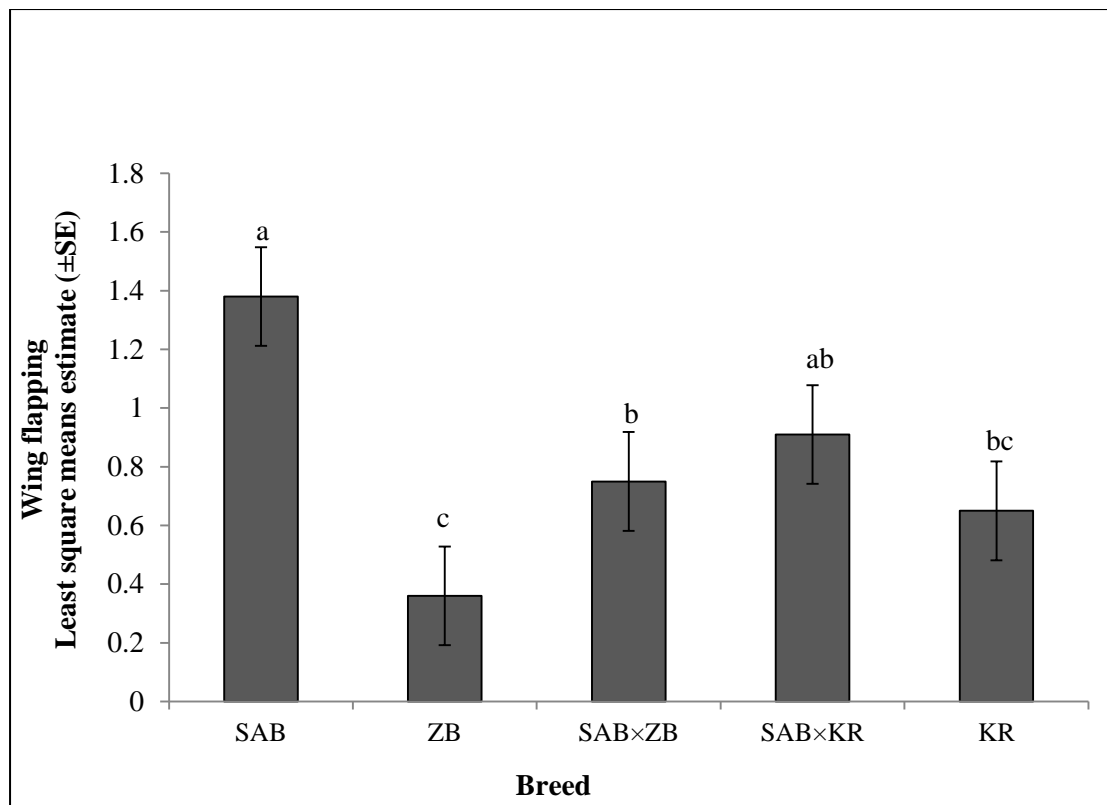


Figure 4.7 The effect of breed on wing flapping by birds that approached the handler (N=207). Means with different superscripts differed significantly ( $P < 0.05$ ). [The breeds were SAB: South African Black (N=160); ZB: Zimbabwean Blue (N=5); KR: Kenyan Redneck (N=4) and their cross-bred combinations: SAB×ZB (N=19); SAB×KR (N=19)].

There was no significant difference in any of the fixed effects on avoiding (keeping a distance from) the handler by birds ( $P > 0.05$ ). However, birds that approached the handlers kept a distance more often when an unfamiliar handler attempted to touch them, compared to a familiar handler ( $1.56 \pm 1.29$  vs.  $4.37 \pm 1.33$ ,  $F_{1, 69} = 4.44$ ,  $P = 0.04$ , respectively). Furthermore, there was a significant effect of sex on excessive pecking, with male birds pecking more excessively at the handler than females ( $F_{1, 1112} = 12.77$ ,  $P = 0.004$ ; Table 4.1). Breed and familiarity of the handler did not have a significance influence on excessive pecking behaviour ( $P > 0.05$ ). Significant differences in hissing were observed between years and breeds ( $P < 0.05$ ). Birds from the 2015 group hissed more often at the handler than the 2013 group ( $F_{1, 1112} = 5.11$ ,  $P = 0.02$ ; Table 4.1). Birds of the SAB breed, KR and SAB×ZB hissed

more often at the handler than the ZB birds (SAB vs. ZB:  $F_{4, 1112}=6.09$ ,  $P=0.001$ ; SAB×ZB vs. ZB:  $F_{4, 1112}=3.03$ ,  $P=0.02$ ; KR vs. ZB:  $F_{4, 1112}=2.60$ ,  $P=0.04$ ; Figure 4.8). None of the experimental birds were observed to kick at the human handler.

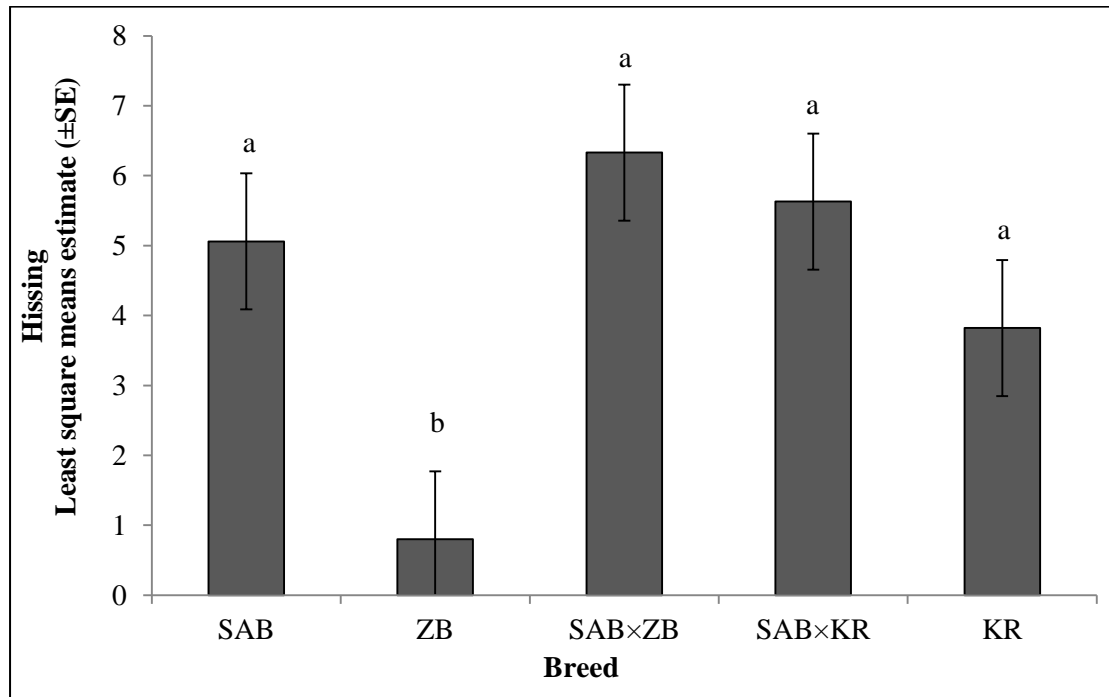


Figure 4.8 The effect of breed on hissing by ostriches that approached the experimenter (N=207). Means with different superscripts differed significantly ( $P<0.05$ ). [The breeds were SAB: South African Black (N=160); ZB: Zimbabwean Blue (N=5); KR: Kenyan Redneck (N=4) and their cross-bred combinations: SAB×ZB (N=19); SAB×KR (N=19)].

Husbandry practices did not influence sexual behavioural responses of male or female ostriches ( $P>0.05$ ). However, male ostriches from the 2015 group stepped and kantled towards the handler more often than the 2013 group ( $F_{1, 622}=12.63$ ,  $P=0.004$  and  $F_{1, 622}=3.86$ ,  $P=0.05$ , respectively; Table 4.2). In addition, stepping was influenced by breed, with SAB ostriches and their cross with KR ostriches (SAB×KR) stepping more regularly towards the handler than KR birds ( $F_{4, 622}=10.75$ ,  $P=0.001$ ).

Table 4.2 Least square mean estimates for sexual behavioural responses of ostriches relative to the year of observation (N=207).

Sexual behavioural response					
Effect	Males			Females	
Year	Clucking	Stepping	Kantling	Clucking	Crouching
2013	6.19±5.38	1.69±0.99	3.66±0.81	6.29±16.47	6.60±8.86
2015	12.5±7.20	4.54±1.13	5.29±0.74	11.60±16.83	9.29±8.77
P-value	>0.05	<0.01	<0.05	>0.05	>0.05

#### 4.3.3 Docility and fear responses

In the docility test, husbandry practice did not have an effect on the time taken to get the birds to enter the marked square or the time the birds were contained in the square area ( $P>0.05$ ). In addition, there was no significant effect of husbandry practice on aggressiveness, vocalization or defecation observed during the test ( $P>0.05$ ). The mean time taken by birds to enter the marked square and the time birds were contained in the marked square amounted to  $49.97\pm3.27$  seconds and  $24.36\pm0.73$  seconds, respectively. The success in containing the birds in the marked square varied significantly with year (2013: 84%; 2015: 25%;  $F_{1,212}=38.71$ ,  $P=0.001$ ). The time taken by the birds to enter the marked square area, the time the birds were contained there and aggressiveness were independent of familiarity, sex, breed and their interactions ( $P>0.05$ ).

In the fear test, the mean distance kept by birds from the stimulus square at the end of Phase 1, Phase 2 and Phase 3 amounted to  $8.97\pm0.63$ m,  $8.77\pm0.81$ m and  $8.83\pm0.79$ m respectively. The number of marked square crossed during Phase 1, Phase 2 and Phase 3 of the fear test was  $2.37\pm0.13$ ,  $2.84\pm0.14$  and  $2.42\pm0.14$ , respectively. The time taken by the

birds to eat, after the handler put feed in the container during Phase 2 and Phase 3, amounted to  $23.00 \pm 0.23$  seconds and  $46.92 \pm 0.17$  seconds, while the time taken to interact with the handler was  $51.95 \pm 0.26$  seconds. There was no effect of husbandry practice on the time taken to eat in Phase 2 and Phase 3 or the time taken to interact with the handler ( $P > 0.05$ ). However, there was a significant difference between Phase 2 and Phase 3 on the time taken to eat ( $23.00 \pm 0.23$  and  $46.92 \pm 0.17$  seconds, respectively;  $F_{1, 20} = 6885.6$ ,  $P = 0.001$ ). There was no effect of familiarity of human handler, sex, breed or the interaction between husbandry practices and sex on the average distance kept by birds from the stimulus square during all phases, the number of square crossed by birds during the test or the time taken by the birds to feed or interact with the handler ( $P > 0.05$ ).

#### 4.4 Discussion

The results of this study revealed that extensive human presence and regular gentle handling at an early age (specifically physical interactions), reduced short- and long-term stress in ostrich chicks. Frequently positively interacted chicks were less sensitive to stress caused by feather harvesting/clipping and routine farm management practices relative to chicks that had limited interactions with humans during early life. This was indicated by the lack of a significant increase in the plasma H/L ratio 72 hours after feather harvesting and feather clipping in the I1 birds compared to I2 and the S birds. Furthermore, while the response of I2 birds was intermediate for long-term stress responses, lower CORT concentrations in the feathers of the I1 birds, as compared to those of the S birds also indicated that I1 birds had lower hypothalamic-pituitary-adrenal axis activation and may reflect lower levels of chronic stress. This is in accordance with previous studies where habituation to human presence and handling has been reported as a potential method to reduce stress associated with routine husbandry practices in farm animals (Hemsworth, 2003; Zulkifli, 2013) including chickens

(Zulkifli et al., 2002; Hemsworth et al., 1994), lambs (Hemsworth, 2003) and dairy cattle (Hemsworth et al., 2000).

The majority of routine farm management practices (i.e. weighing, vaccination, dosing, blood sampling) are generally stressful, as animals need to be physically restrained (Waiblinger et al., 2006). Although some of these management practices can be considered as minimally invasive or non-invasive, animals not habituated to human handling may be more stressed or suffer from constant fear of human presence (Hemsworth, 2003) than habituated animals. Ostrich chicks may experience various actions as stressors; such as translocation from one camp to another, being mixed with unfamiliar chicks (even of the same age) and by the sudden absence of a familiar human if chicks were reared using the extensive human presence method (Tenessen, 1989; Kamau et al., 2002; Hoffman and Lambrechts, 2011). In this study, ostrich chicks reared using extensive human presence and regular gentle handling appeared to be less sensitive to long-term stress caused by routine farm management practices (as indicated by their lower CORT levels in compared to chicks reared with limited human presence). These results also suggest that application of positive regular gentle handling for longer periods after hatching may have accelerated the ability of ostrich chicks to adapt to farm management practices which consequently reduced their sensitivity to stressful events.

Interestingly, no significant differences were observed between husbandry practices in terms of the birds' willingness to approach the handler, to allow for touch interactions, to display aggressiveness or sexual behavioural responses, or in the docility and fear tests. This is in contradiction with studies on dairy cows (Breuer et al., 2003), pigs (Tanida et al., 1994; de Oliveira et al., 2015; Somnavilla et al., 2016) and chickens (Jones and Faure, 1981; Hemsworth et al., 1994). These studies reported that animals exposed to human presence and regular gentle handling approached a human handler more frequently than those that received



limited human presence. A possible explanation of the lack of differences between treatments in the present study is that chicks may have familiarized themselves with humans even when human presence was limited to provision of feed and fresh water and routine farm management operations. Furthermore, the lack of a significant effect of husbandry practices during the docility and fear tests may be explained by two principles; firstly, the birds could have been stressed when caught by an unfamiliar catching crew during the tests or due to isolation from their peers (Waiblinger et al., 2006); secondly, the tests were not strict enough to differentiate between birds on the basis of early-life experiences. Frequent vocalization and/or excessive urination or defecation has been reported as behavioural responses exhibited by stressed animals (Hemsworth et al., 2011; Bejaei and Cheng, 2014), most especially those under isolation (Waiblinger et al., 2006). The lack of such signs of stress from the birds in this study might corroborate that the tests were not strict enough to differentiate between birds on the basis of early-life experiences.

Birds exposed to extensive human presence were found to be more likely to approach a familiar rather than an unfamiliar handler although they were wearing similar clothing. The concept of livestock animals discriminating between familiar and unfamiliar human handlers is not uncommon (chickens: Davis and Taylor, 2001; pigs: Tanida and Nagano, 1998; Koba and Tanida, 2001; cattle: Munksgaard et al., 1999; Breuer et al., 2003) and has been reported to benefit animal welfare (Hemsworth, 2003; Tallet et al., 2018). Generally, a relationship is established between individuals that are familiar to each other. For example, cows were able to distinguish between people wearing differentially-dyed clothing but could not differentiate between people wearing clothing of the same colour (Munksgaard et al., 1999; Rushen et al., 1999). The preference for a familiar human handler by animals can be interpreted as a lack of fear of the familiar handler and may indicate that the welfare of an animal is not as affected when handled by a preferred or familiar human.

Interestingly, a higher success rate for the docility test was obtained in the 2013 birds compared to the 2015 birds. Birds hatched in 2013 were also contained for a longer period of time in the marked square compared to the 2015 birds. This may be attributed to the level of experience of the handlers which in 2013 was greater than in 2015. Previous studies have reported that the attitude or experience of stockpeople may affect animal handling and productivity (Lensink et al., 2000; Hemsworth and Coleman, 2011; Coleman and Hemsworth, 2014). For example, stockpeople with a negative attitude or less handling experience of animals under their care are most likely to handle animals in a more stressful way than experienced stockpeople (Coleman and Hemsworth, 2014). These results suggest that training and selection of stockpeople based on their competency and work ethics may be a priority to improve animal welfare in the ostrich industry.

The results of the fear test showed that the latency of birds to feed during Phase 2 when the handler was not in the test arena was longer than in Phase 3. Moreover, the birds were more likely to feed directly from the feed container close to the observer, than to interact with the handler in Phase 3. Hemsworth et al. (1996) reported that the initial contact between humans and animals is important in establishing subsequent behavioural responses towards humans. Therefore the delayed interactions with the human in this trial may suggest that the birds associated the handler with prior handling by the person taking them from the holding pen and introducing them to the test arena. Based on these results further studies may be warranted to refine methods of assessing docility and fear in ostriches. Given the assumptions that docile animals are calm and less nervous while flighty animals are more agitated when nervous, the evaluation of the flight distance kept by birds after handling as described by Burrow et al. (1988) might be needed to estimate docility and fear responses in ostriches more accurately. Furthermore, the results of such studies could assist in the

selection of docile birds that show less fear of humans during routine husbandry practices (e.g. tagging, weighing).

#### 4.5 Conclusions

The results of this study demonstrated that extensive human presence and regular gentle handling of ostrich chicks at an early age reduced short- and long-term stress sensitivity later in life. Chicks exposed to extensive human presence and regular gentle handling had lower levels of physiological stress indicators than chicks exposed to limited human presence. While the willingness of birds to associate with a human was not affected by husbandry practices, birds habituated to human presence and regular gentle handling as chicks were able to distinguish between a familiar and unfamiliar handler, and adjust their behaviour accordingly. This result suggests that ostriches may be easier to manage when handled by a familiar human. There was no effect of husbandry practices on the tested docility and fear responses. This may indicate that the tests used were inadequate to reveal/uncover any differences in behaviour of birds subjected to different levels of human interaction and presence at an early age. Consequently, this altered state prevented expression of differences in behaviour that can be used to associate them with prior human experience. Further studies on assessing fear and docility of ostriches are therefore still needed. The difference in experience of ostrich handling and management between the 2013 and 2015 handlers during the docility test also raise concerns regarding the training of stockpersons.

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## **Chapter 5**

# **Egg production in ostriches is improved by positive human-bird interactions performed at an early age, but not meat quality and skin damage**

**P.T. Muvhali., M. Bonato., A. Engelbrecht., I.A. Malecki., C. Mapiye & S.W.P. Cloete**

## Abstract

Positive human-animal interactions are known to improve productivity in several commercial livestock industries, but there is currently no evidence that this is the case in ostriches. This study was therefore conducted to evaluate the effect of human presence and regular gentle handling performed at an early age on meat quality, skin damage and egg production. A total of 416 day-old chicks were exposed to three different husbandry practices for three months over two breeding seasons. Husbandry practices differed in human presence and human-chick interactions (I1: extensive human presence with audio, visual and touch stimuli; I2: extensive human presence with audio and visual stimuli; S: human presence limited to feed and water supply). Meat quality was evaluated post slaughter by measuring meat pH, colour, tenderness and proximate composition on a sample of 24 1-year old South African Black (SAB) ostriches, 4 males and 4 females from each husbandry practice. Skin damage from the slaughtered birds was evaluated by quantifying the number of lesions on the skin surface. Finally, female reproductive performance was evaluated over two successive breeding seasons on a sample of 14 pair mated SAB ostriches (I1: N=7; S: N=7). Husbandry practices did not have an effect on meat pH, colour and skin damage ( $P>0.05$ ). Overall egg production and number of eggs per clutch was higher for I1 females compared to S females ( $P<0.05$ ). Moreover, I1 females produced a greater number of eggs during their first breeding season than S females ( $P<0.05$ ), but no significant difference was recorded during the second year of production ( $P>0.05$ ). Current results suggest that, although extensive human presence and gentle handling at an early age did not have a direct effect on meat pH, meat colour and skin damage, it did facilitate the adaptation of females to the pair breeding environment as demonstrated by their higher egg production performance during their first year of breeding.

## 5.1 Introduction

South Africa is considered the leader in ostrich production globally, contributing about 70% of all the ostrich products worldwide (Brand and Jordaan, 2011). The ostrich industry is mainly based on the production of meat, leather and feathers, with meat and leather contributing the most to slaughter income. Ostrich meat has been demonstrated as a rich source of protein and contains considerably low levels of cholesterol, compared to beef and chicken (Poławska et al., 2011; Al-Khalifa and Al-Naser, 2014), while leather derived from ostrich skins is considered unique, durable and attractive to the fashion industry (Engelbrecht et al., 2009). The market of ostrich products from South Africa is based almost entirely on exports to the European Union (EU), with only a small portion marketed locally (Brand and Jordaan, 2011). However, the EU maintains strict standards regulating the import of food materials, which is further driven by consumers concerns and willingness to pay more for products derived from animals that were treated humanely (Miranda-de la Lama et al., 2017). Hence, the ostrich industry should align with the EU recommendations for animal welfare and product quality to remain competitive in this market.

Previous studies on ostriches have revealed that pre-slaughter factors such as transportation, a novel abattoir environment and lairage are stressful to the birds and affect meat quality (Van Schalkwyk et al., 2005; Hoffman and Lambrechts, 2011; Wolmarans, 2011; Hoffman et al., 2012). Animals experiencing long-term stress before slaughter secrete glucocorticoid hormones such as cortisol which results in the depletion of muscle glycogen, a higher meat pH and dark, firm and dry meat (Hemsworth et al., 2011; Chulayo et al., 2012). On the other hand, short-term stress just before slaughter potentially leads to a lower meat pH. This is due to the rapid conversion of glycogen to lactic acid, which results in pale, soft and exudative meat (Terlouw, 2005; Adzitey and Nurul, 2011). Thus, both these extremes of high and low meat pH would result in meat of poor quality (Adzitey and Nurul, 2011). While

stress caused by transportation of ostriches prior slaughter may be reduced by improving driving skills (Hoffman and Lambrechts, 2011; Wolmarans, 2011), birds not habituated to human presence may also encounter stress caused by human presence and contact at the abattoir. Hence, early habituation of ostriches to human presence and care may reduce stress in abattoir conditions and potentially improve meat and leather quality.

The concept of habituating animals to humans through positive interactions has increasingly gained attention in various livestock industries. It was shown to benefit both production and animal welfare, when practised appropriately (cattle: Rushen et al., 1999; Hemsworth et al., 2000; pigs: Hemsworth et al., 1994; poultry: Barnett et al., 1994). For example, when exposed to an aversive handler during milking, previously negatively handled cows exhibited a reduced milk production, lower milk protein and fat concentrations, as well as an impaired milk let-down reflex (Rushen et al., 1999; Hemsworth et al., 2000). This resulted in an increased milk residual compared to previously positively handled cows. In pigs, sows that received positive handling procedures produced more piglets per litter than negatively handled sows (Hemsworth et al., 1994). Similarly, housed White Leghorn layers that received regular human presence had an increased egg production, compared to hens receiving minimal human presence (Barnett et al., 1994). In ostriches, several studies have been conducted on production traits in a commercial farming environment (Cloete et al., 1998; 2004; 2008; 2012; Bunter, 2002, Engelbrecht et al., 2009; Cloete and Brand, 2014; Bonato et al., 2017), but these birds were all reared using standard practices with limited human-bird interactions. Although rearing ostrich chicks using extensive human presence and gentle handling has been shown to improve welfare and stress-coping mechanisms in the sensitive stage of their lives (Chapter 3 and Chapter 4), there is still no evidence on the impact of such a practice on ostrich production traits. Thus, the aim of this study was to

evaluate the effect of extensive human presence and regular gentle handling during rearing on meat quality, skin damage and egg production performance.

## 5.2 Materials and methods

### 5.2.1 Sampling population and study area

The sampling population, the study area and husbandry practices have been previously described in Chapter 3, while the management of the breeding stock from which the chicks used in this study originated from has been described previously by Bunter and Cloete (2004) and Cloete et al. (2008). Briefly, a population of 416 day-old chicks were exposed to three different husbandry practices, which varied in human presence and human-chick interactions. The husbandry practices comprised of extensive human presence with visual, audio and touch stimuli (I1), extensive human presence with only visual and audio stimuli (I2) and the standard husbandry practice used to rear chicks at the Oudtshoorn Research Farm where human presence is limited to the supply of feed and fresh water (S). Groups of chicks hatched during the 2013 and 2015 breeding seasons, respectively, were exposed to these husbandry practices for 3 months post hatching, after which all chicks were grouped together with standard care provided from there on onwards. Ethical clearance to conduct this study was granted by the Western Cape Department of Agriculture's Departmental Ethical Committee for Research on Animals (Ref No.: R13/81.).

### 5.2.2 Meat quality, carcass attributes, skin damage and egg production performances

To study the effect of extensive human presence at an early age on ostrich meat quality at slaughter, 24 1-year old SAB ostriches (2015: 4 males and 4 females from each husbandry practice) were slaughtered at 12 months of age. The birds were transported to a commercial abattoir in Oudtshoorn (Klein Karoo International PTY LTD) less than 10 km away from the

study location a day before slaughter. At the abattoir the birds were kept overnight in roofed kraals and supplied with only fresh clean drinking water until the following morning. The birds were then weighed and slaughtered following the routine standard slaughter procedures for an EU approved commercial abattoir (South African Ostrich Business Chamber, 2011).

Initial meat pH and temperature were measured 45 minutes after slaughter ( $\text{pH}_i$  and  $\text{temp}_i$ ), while final measurements were taken 24 hours after slaughter ( $\text{pH}_f$  and  $\text{temp}_f$ ) on the left big drum muscle (*M. gastrocnemius*) using a portable pH meter (Cormark) and digital thermometer (Comark PDQ 400), respectively. Hot carcass weight was recorded 45 minutes post slaughter while cold carcass weight was determined 24 hours post slaughter. Dressing percentage was calculated as the percentage of cold carcass weight to initial slaughter weight.

The right drumstick was excised and weighed, from which the big drum (*M. gastrocnemius*) and fan fillet (*M. iliofibularis*) muscles were removed for further analyses. These muscles were selected as they are the largest single cuts and are commonly available in the market (Mellett, 1985). Meat colour of these muscles was determined objectively using the Colour-guide 45°/0° colorimeter (Catalogue No: 6805; BYK-Gardner, USA) by recording the  $L^*$ ,  $a^*$  and  $b^*$  values (where:  $L^*$  is the lightness;  $a^*$  the red-green range and  $b^*$  the blue-yellow range). The  $L^*$ ,  $a^*$  and  $b^*$  values were used to calculate the hue angle and chroma of individual muscles. Meat tenderness of these muscles was determined as shear force, using the Warner Bratzler shear force attachment, fitted to an Instron Universal Testing Machine (Model 4444). Small pieces of 1.5cm thickness from each muscle were cut perpendicular to the longitudinal axis of the muscle. The Warner Bratzler shear force values required to shear the 1.5cm cooked meat sample were expressed in kilograms (kg).

The skin of the birds was identified with a microchip that was attached to each skin and linked to the slaughter number before being taken to the tannery. All skins were cured

and processed using similar tannery treatment. After processing, the skins were graded by qualified ostrich skin graders. Skin grades were allocated according to the location of lesions/defects present in the crown area, as recommended by the National Processors of South Africa grading standards described by Meyer (2003). Hereafter, the processed skins were brought to the research facility and the number of lesions on the skins was quantified as an indication of skin damage. The incidence of feather pecking as indicated by damage to the feather follicles as a result of pecking was also recorded. Skin thickness (mm) was measured and represented as an average of three separate measurements on the right hand side of the skin, outside of the crown area using a caliper (Engelbrecht, 2013).

To evaluate the effect of husbandry practices on egg production performance, a total of 14 two-year old pair-mated SAB ostriches (hatched in 2013: 7×I1 and 7×S) were randomly allotted to the pair breeding paddocks for two consecutive breeding seasons (2015 and 2016). Due to a lack of camp availability, only I1 and S birds were selected to compare the extreme husbandry practices performed in this study. The males used for mating were of the same age as their paired females and had experienced similar husbandry practices. The eggs produced by these pairs were collected twice a day (morning and afternoon), identified according to camp of origin, weighed using an electronic weighing balance (Precisa, XT 4200 C) and incubated in electronic incubators until hatching. During the 42-day incubation period, eggs were candled on day 21 to evaluate embryo development (Brand et al., 2013). The development of an embryo indicated whether or not the egg was fertilised. The number of eggs laid by each female, number of clutches and number of eggs per clutch were subsequently calculated for all breeding seasons. A clutch was defined as any sequence of eggs laid within four days of each other, as female ostriches typically lay an egg every second day (Bunter, 2002). Upon incubation and hatching the number of chicks hatched per female was recorded, as well as the average chick weight expressed as a trait of the female.



### 5.2.3 Statistical analysis

A 3×2×2 factorial design was used to evaluate the effect of husbandry practices, sex, and muscle type on ostrich meat quality. Using the General linear models procedure, carcass and meat quality traits were entered as dependent variables, while husbandry practices, sex, muscle type and their interactions were entered as fixed effects. Bird identity was entered as a random variable to account for the repeated sampling of the same animal. To evaluate the effect of husbandry practice on skin damage, the number of lesions present on the skin surface and skin grade were entered as dependent variables, as well as skin weight and skin thickness. The data was analyzed in a Generalized Linear Mixed Model (GLMM) with husbandry practice, sex and their interaction entered as fixed factors. A similar GLMM was performed on the effect of husbandry practice on feather pecking, with the binomially distributed dependent variable (presence/absence of feather pecking or sunburn) normalized using the logit link function. Least square means of these factors together with their interactions were generated during the analysis and recorded with the standard errors. Finally, the effect of husbandry practices on female production performance was assessed by fitting a GLMM with the number of eggs produced, mean egg weight per female, number of clutches, number of eggs per clutch, number of chicks produced and mean chick weight per female entered as dependent variables. Husbandry practices and year of production was entered as fixed factors; and breeding paddock was entered as a random variable. The fertilisation status of individual egg records at candling (1: fertilised/embryo; 0: not fertilised/no embryo development) was analysed using a similar GLMM as that for female production performances. The factors were considered to be statistically significant at  $P < 0.05$ . All analyses were performed using SAS, version 9.3 (SAS, 2012).

### 5.3 Results

The overall means ( $\pm$ SEM) for  $\text{pH}_i$  and  $\text{pH}_f$  after slaughter was  $5.73 \pm 0.07$  and  $5.48 \pm 0.03$ , respectively. Slaughter weight, cold carcass weight, drumstick weight and dressing percentage accordingly were recorded as  $98.60 \pm 2.25\text{kg}$ ,  $45.67 \pm 0.91\text{kg}$ ,  $17.16 \pm 0.28\text{kg}$  and  $46.72 \pm 1.18\%$ , respectively. Neither husbandry practice, sex nor the interaction between these factors had a significant effect on slaughter weight,  $\text{pH}_i$ ,  $\text{pH}_f$ , cold carcass weights, drumstick weight or dressing percentage ( $P > 0.05$ ; Table 5.1).

Table 5.1 Least square means and standard error (SEM) for ostrich slaughter traits (slaughter weight,  $\text{pH}_i$ ,  $\text{pH}_f$ , cold carcass weights, drumstick weight and dressing percentage) according to husbandry practices varying in the degree of human presence and human-bird interaction.

Physical meat traits	Husbandry practice			SEM	P-value
	I1	I2	S		
Slaughter weight (kg)	102.1	98.8	94.9	3.97	$>0.05$
$\text{pH}_i$	5.85	5.61	5.71	0.06	$>0.05$
$\text{pH}_f$	5.54	5.43	5.47	0.03	$>0.05$
Cold carcass weight (kg)	44.7	44.5	44.8	0.90	$>0.05$
Hot carcass weight (kg)	46.09	48.58	46.50	1.50	$>0.05$
Drumstick weight (kg)	17.3	17.3	16.8	0.28	$>0.05$
Dressing (%)	44.2	48.1	47.8	2.13	$>0.05$

The overall lightness ( $L^*$ ), redness ( $a^*$ ), yellowness ( $b^*$ ), hue angle ( $^\circ$ ) and chroma amounted to  $30.47 \pm 0.28$ ,  $15.06 \pm 0.19$ ,  $7.82 \pm 0.21$ ,  $27.44 \pm 0.76^\circ$  and  $17.03 \pm 0.18$ , respectively. There was no significant effect of either husbandry practices or sex on any of the meat colour traits ( $P > 0.05$ ). The overall moisture, dry matter, ash, lipid and protein percentages of the meat

recorded were  $74.18 \pm 0.33\%$ ,  $25.82 \pm 0.33\%$ ,  $1.41 \pm 0.14\%$ ,  $1.93 \pm 0.08\%$  and  $23.41 \pm 0.32\%$ , respectively. There was no significant effect of husbandry practices on the percentage moisture, dry matter, ash, lipid or protein percentage of the meat ( $P > 0.05$ ). However, there was a significant interaction between husbandry practices and muscle type for moisture, dry matter and protein percentage of the meat ( $P < 0.05$ ; Table 5.2). The big drum muscle of the I1 birds had a lower moisture percentage compared to the fan fillet of I1 and I2 and the big drum of S ( $P < 0.05$ ). Also, the big drum of I1 birds recorded a higher dry matter and protein percentage than the big drum and fan fillet of I2 birds and the big drum of S birds, but not the fan fillet of S birds ( $P < 0.05$ ). Furthermore, there was a significant effect of muscle on lipid composition ( $P < 0.05$ ). The percentage of lipid was higher in the fan fillet ( $2.17 \pm 0.09\%$ ) than in the big drum ( $1.69 \pm 0.09\%$ ;  $P < 0.01$ ). Husbandry practice, sex, muscle and their interactions had no effect on the ash content of the meat ( $P > 0.05$ ).

Table 5.2 Least square means and standard error (SEM) for proximate composition (moisture, dry matter and protein content), representing the interaction between muscle and husbandry practices (I1, I2 and S) for proximate traits.

Proximate	I1		I2		S		SEM
characteristic	Big drum	Fan fillet	Big drum	Fan fillet	Big drum	Fan fillet	
Moisture (%)	72.1 <sup>b</sup>	74.9 <sup>a</sup>	74.7 <sup>a</sup>	74.6 <sup>a</sup>	74.9 <sup>a</sup>	73.9 <sup>ab</sup>	0.73
Dry matter (%)	27.8 <sup>a</sup>	25.0 <sup>b</sup>	25.3 <sup>b</sup>	25.4 <sup>b</sup>	25.1 <sup>b</sup>	26.1 <sup>ab</sup>	0.73
Protein (%)	25.5 <sup>a</sup>	22.3 <sup>b</sup>	23.0 <sup>b</sup>	22.7 <sup>b</sup>	23.3 <sup>b</sup>	23.7 <sup>ab</sup>	0.73

<sup>a,b</sup> Means with different superscripts across the rows differed significantly ( $P < 0.05$ ).

The overall shear force as a measure of meat tenderness was recorded as  $6.90 \pm 0.18\text{kg}$ . There was no significant effect of husbandry practice on meat tenderness ( $P > 0.05$ ). However, a significant interaction between sex and husbandry practice for meat tenderness was observed

( $P < 0.05$ ). Shear force was higher for males from the I1 husbandry practice ( $7.77 \pm 0.42 \text{ kg}$ ), compared to that of males from the S husbandry practice ( $6.28 \pm 0.42 \text{ kg}$ ). Furthermore, females from the I1 husbandry practice had a lower shear force ( $6.26 \pm 0.42 \text{ kg}$ ) than males from the same group ( $7.77 \pm 0.42 \text{ kg}$ ).

The overall mean skin size, skin weight and skin thickness was  $144.33 \pm 0.94 \text{ dm}^2$ ,  $1.05 \pm 0.03 \text{ kg}$  and  $0.78 \pm 0.03 \text{ mm}$ , respectively. There was a significant difference between husbandry practice and sex for skin weight when live weight was not accounted for in the model ( $P < 0.001$ , Table 5.3). The skins from the S birds were the heaviest, while those from I2 were intermediate and I1 the lightest. Skins from males were also heavier than those of the females ( $P < 0.001$ ). The number of lesions, skin grading, skin size, skin thickness and the presence of feather pecking were not significantly influenced either by husbandry practice, sex, or the interaction between husbandry practice and sex ( $P > 0.05$ ).

Table 5.3 Least square means ( $\pm \text{SEM}$ ) for the effect of ostrich husbandry practice and sex on skin weight (kg).

Skin trait	Husbandry practice			Sex	
	I1	I2	S	Male	Female
Skin weight (kg)	$1.04 \pm 0.02^a$	$1.06 \pm 0.02^b$	$1.07 \pm 0.02^c$	$1.07 \pm 0.02^d$	$1.04 \pm 0.02^e$
P-value	$< 0.001$			$< 0.001$	

<sup>a b c</sup> Least square means for husbandry practices with different superscripts differ significantly ( $P < 0.001$ ).

<sup>d e</sup> Least square means for sex with different superscript differed significantly ( $P < 0.001$ ).

Husbandry practice: I1 (N=8), I2 (N=8) and S (N=8)

The overall number of eggs, mean egg weight (g), number of clutches and number of eggs per clutch recorded were  $50.43 \pm 4.11$  (range: 4-93 eggs),  $1400 \pm 25.84 \text{ g}$  (range: 1162-1729g),

6.57±0.78 (range: 2-19) and 12.01±2.25 (range: 1-46.5), respectively. The number of chicks hatched and mean chick weight (g) recorded was, 22.54±3.72 (range: 0-66 chicks) and 860.92±18.54g (range: 746-1036g), respectively. There was a significant difference between husbandry practices for the overall number of eggs produced ( $P<0.05$ ). I1 females produced significantly more eggs compared to S females (Table 5.4). Furthermore, I1 females produced on average a higher number of eggs per clutch, compared to the S group of females ( $P<0.05$ , Table 5.4). Husbandry practice did not have an effect on the overall egg weight, number of clutches, number of chicks hatched and chick weight ( $P>0.05$ ). In addition, the year of production (first and second year of production) did not have an effect on the overall number of eggs laid, egg weight, number of clutches, number of eggs per clutch, number of chicks hatched and chick weight ( $P>0.05$ ), respectively.

Table 5.4 Least square means ( $\pm$ SE) for the number of eggs laid, mean egg weight (g), number of clutches and number of eggs per clutch of ostriches relative to husbandry practice.

Reproduction traits	Husbandry practice		P-value
	I1	S	
Number of eggs laid	58.85±5.49	42.00±5.49	<0.05
Egg weight (g)	1429.36±35.67	1372.14±35.67	>0.05
Number of clutches	6.71±1.16	6.65±1.21	>0.05
Number of eggs per clutch	14.55±2.48	9.69±2.48	<0.05
Number of chicks hatched	26.00±5.04	19.07±5.04	>0.05
Chick weight (g)	878±26.16	838.78±28.47	>0.05

Husbandry practices: I1 (N=7) and S (N=7).

During the first year of entering the breeding flock (i.e. 2 year old), I1 females produced significantly more eggs compared to S females ( $P<0.05$ , respectively; Figure 5.1), while there

was no difference between husbandry practices during the second year of production ( $P>0.05$ ). Mean egg weight, number of clutches, number of eggs per clutch, number of chicks hatched and mean chick weight did not differ significantly between the ostrich husbandry practices for the first and second year of production ( $P>0.05$ ). There were no differences in egg production (Figure 5.1), mean egg weight, number of clutches, number of eggs per clutch, number of chicks hatched and mean chick weight for the I1 group of birds between the first and second breeding season ( $P>0.05$ ).

Furthermore, no differences were recorded in egg production (Figure 5.1), mean egg weight, number of clutches, number of eggs per clutch, number of chicks hatched and mean chick weight for the S birds between the first and the second breeding season ( $P>0.05$ ). There were also no significant differences between husbandry practices for the egg fertility rates and the overall production for each breeding season ( $P>0.05$ ). However, the fertility rates of the S birds improved significantly during the second breeding season ( $P<0.05$ ). On one hand, the proportion of fertilised eggs produced by the S birds (limited human presence) during the first breeding season was 63.03% while it was 74.33% during the second breeding season ( $P<0.05$ ). On the other hand, the proportion of fertilised eggs produced by the I1 birds did not differ significantly between the two breeding seasons (64.5% and 66.8%;  $P>0.05$ ).

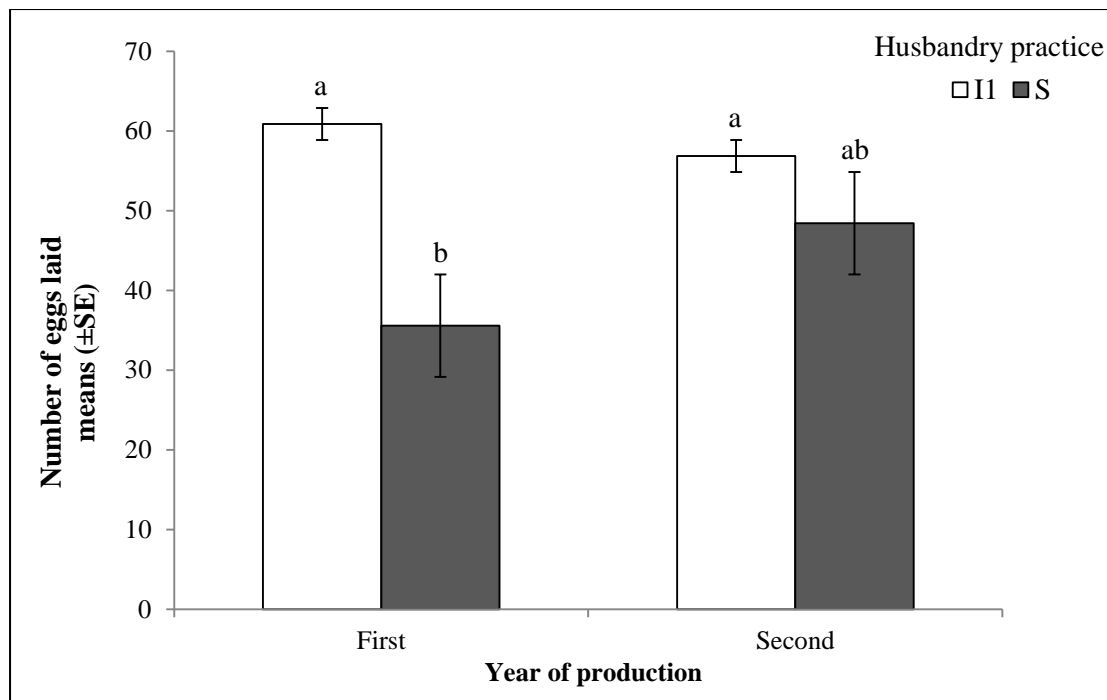


Figure 5.1 Egg production performances of ostrich females for the I1 and S husbandry practices during the first and second breeding year. Means with different superscripts differ significantly ( $P < 0.05$ ). Husbandry practices: I1 (N=7) and S (N=7).

#### 5.4 Discussion

The results of this study revealed that the husbandry practices of varying degrees of human exposure did not have an effect on ostrich meat pH, colour or skin damage at slaughter. However, ostriches habituated to human presence at an early age produced more eggs, as well as more eggs per clutch during their first breeding season. Fertility rates did not differ between the two husbandry practices, but birds that received limited human presence at an early age had a lower fertility rate during their first year of breeding than in the second year of breeding.

The lack of an effect of early human exposure on meat pH, colour and carcass characteristics in the current study is consistent with previous findings regarding veal calves and pigs (Lensink et al., 2000; Terlouw et al., 2005). Meat quality traits of veal calves (Lensink et al., 2000) and large white pigs (Terlouw et al., 2005) was not affected by positive

or negative interactions with humans. Factors such as transportation, novel environment and unusual noise have been reported to be stressful to animals and affect meat pH after slaughter (Chulayo et al., 2012). For instance in cattle, beef from bulls that had previous experience to transportation before been transported to the abattoir for slaughter had a lower pH, compared to that of bulls that were only transported once for slaughter (Mounier et al., 2008).

In the present study, the routine management of birds involved previous experience to transportation to a similar extent (i.e. transported from the chick rearing facility to the feedlot facility). This short transportation distance may have familiarize the birds to transportation, thus reduced the potential pre-slaughter stress. Furthermore, the distance ostriches were transported for slaughter purposes have been revealed to influence meat pH post-mortem. Ostriches transported for 60km showed pH-values of 6.30 1 hour and 5.93 24 hours after slaughter. Transportation for 600km accordingly resulted respective in values of 6.37 and 6.11 (Hoffman et al., 2012). Although the birds in this study were transported for less than 10km to the abattoir, their pH-values corresponded well with those reported by Hoffman et al. (2012) for birds that were not transported to the abattoir before slaughter (pH 1 hour=5.8 and pH 24 hours=5.77). This could account for the lack of differences between husbandry practices, as birds seems to have responded to the same extent to transportation.

Furthermore, the lack of differences between husbandry practices in meat pH, colour and carcass attributes in the current study may indicate that the pre-slaughter stress experienced by birds was not strong enough to elicit a response that correspond to early habituation to humans. In this study, females exposed to extensive human presence and regular gentle handling produced meat with a lower shear force, compared to males from the same group. This may indicate that the males experienced more short-term stress before slaughter than females. The short-term stress in males may have activated higher concentrations of lactic acid, leading to tougher meat (Grandin, 1980). While birds from the



other husbandry practices might have experienced similar short-term stress, the lack of differences and the small number of birds slaughtered in this study suggest future investigations using larger numbers, as well as behavioural observations under abattoir conditions.

Habituation to human presence has been alleged to potentially cause problems related to the production success of ostriches (Bubier et al., 1998). Ostrich mating is commonly preceded by a courtship display during which males perform kantling and stepping repertoires to females, with females typically responding by clucking and crouching (Bolwig, 1972; Bubier et al., 1998; Malecki and Rybnik-Trzaskowska, 2011). Bubier et al. (1998) reported that ostriches habituated to humans may direct their courtship behaviour more frequently towards humans than towards their mates, thus potentially compromising their success of reproduction. The results of the current study refutes this argument as ostriches that received extensive human presence and care at an early age produced an overall higher number of eggs, with more eggs per clutch compared to birds that were reared with minimal human care (S-group). The former birds also produced a higher number of eggs during their first year of production compared to the latter, although the difference diminished during the second year of breeding. Furthermore, females that received extensive human presence and care at an early age had similar egg fertility rates in both the first and second breeding year. However, females that received limited human presence had a lower fertility rate during the first year of production than the second year.

The results of this study are consistent with previous studies on chickens (Barnett et al., 1994) and dairy cows (Bertenshaw et al., 2008) that revealed improved production and facilitation of adaptation to management practices by habituation to human presence. Extensive human presence and care in ostriches may facilitate the adaptation of female ostriches not only to the stress and agitation caused by the human presence imposed during

daily inspection/egg collection, but also to the pair breeding environment. Hemsworth (2007) argued that agitated/stressed animals may divert the nutrients/substrates designated for production elsewhere, thus compromising their production. This could possibly explain the lower egg production observed in the S females possibly because of the time they needed to adapt to a new environment. Studies investigating the behaviour of both I1 and S birds in the pair breeding environment, as well as in bigger flock situations, are required to confirm or refute this allegation. Furthermore, the recording of the production performances of birds exposed to the S and I1 practices during the 2015 breeding season is currently underway to evaluate the robustness of the pattern observed. Finally, as the low egg fertility rates from S females on the first breeding season could be an indication of late maturity for S males, further studies evaluating egg fertility after laying, and the number of sperm that reached the egg could shed light on the difference observed between husbandry practices (Malecki et al., 2004).

## 5.5 Conclusions

Habituating ostrich chicks to human presence and gentle handling up to 3 months of age after hatch did not significantly influence their meat or skin quality at slaughter. Female ostriches that were exposed to extensive human presence and gentle handling produced a higher number of eggs during their first year of production, compared to ostriches that experienced limited human presence. Although there were no significant differences in egg production during the second year of breeding, these results suggested that extensive human presence and regular gentle handling at an early age may have facilitated quicker adaptation of I1 female ostriches to the pair breeding system relative S females. Further studies to evaluate the egg production performances of I1 and S females during their entire breeding lifespan and their behaviour in the breeding environment as well as male age at maturity could be

important to explain lower fertility rates of eggs from S females during their first year of breeding.

## 5.6 Acknowledgements

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## **Chapter 6**

### **General conclusions and recommendations**

This study investigated the effects of integrating positive interactions between human handlers and ostrich chicks not only for welfare but also for production performance in this species. Specifically it highlighted that slightly changing those management practices currently in place, as well as human-animal interactions with this recently domesticated farmed bird species could overcome major constraints faced by ostrich farmers. Specific components of importance to the wellbeing of and outputs yielded by the birds were thus considered.

### 6.1 Growth, survival and immune competence of chicks

Ostrich chicks reared using extensive human presence and regular gentle handling appeared to have had an improved survival rate and weight gain during their most sensitive period of life (0-3 months of age), compared to chicks that had human presence limited to the provision of food and water. These results suggested that such human-bird interactions may have played a nurturing role towards the chicks and prompted them to feed at an early age, subsequently reducing their chances of starvation and death. Also, extensive human presence in the chick house could have acted as a continuous inspection, allowing the rapid detection of chicks showing unusual conditions and subsequently getting treated before their conditions got critical and life-threatening.

Ostrich chicks that were exposed to extensive human presence and regular gentle handling (specifically physical interactions) also appeared to have developed a stronger immune competence, compared to chicks that received a limited human presence. This was revealed by a lower proportion of positive Hemagglutination-Inhibition titers exhibited by the latter chicks after vaccination against Newcastle disease virus. It also appeared that the former chicks required a lower dilution rate to cause a complete inhibition of an antigen, compared to chicks that received minimal human presence and care. As ostrich chicks are

prone to diseases, building-up a stronger immune competence at an early age is important since it was previously shown to also influence chick growth (Bonato et al., 2009).

## 6.2 Short- and long-term stress responses to routine farm management practices

Raising farm animals using intensive husbandry practices has been revealed to be stressful, due to frequent interactions between humans and animals. However, deliberate positive human-animal interactions have been documented in several developed livestock industries as a method to alleviate stress caused by handling, farm management practices and general human presence. Feather harvesting and feather clipping methods were used to potentially initiate short-term stress in the chicks studied. An overall increase in heterophil/lymphocyte (H/L) ratio 72 hours after feather treatments was observed, suggesting that both these procedures were stressful to the ostriches. However, birds that received extensive human presence and regular gentle handling (specifically physical handling) appeared to be less sensitive to the short-term stress caused by both feather harvesting and clipping. There was no significant increase in the H/L ratio recorded for these birds. This result was also confirmed over the longer term by lower feather corticosterone levels recorded in these birds, when compared to that of birds which received minimal human presence and care. Corticosterone is generally deposited in the feathers over a long period of time during growth when chicks experience stressful events. Therefore, it is a good indicator of long-term stress responses. All these results suggest that extensive human presence and regular gentle handling reduced short- and long-term stress sensitivity experienced by ostrich chicks during rearing. This could in turn explain the higher weight gain and survival rates also observed in chicks exposed to such husbandry practices.

### 6.3 Docility, fear responses and preferences of familiar human contact

The relatively wild behaviour of ostriches makes the management of these birds particularly difficult and dangerous not only to themselves but also to stockpeople working with them. Other livestock industries have integrated positive human-animal interactions within the day-to-day management to facilitate the ease of handling. In this study, extensive human presence and regular gentle handling did not have an effect on the birds' social behavioural responses. However, such husbandry practices appeared to stimulate the willingness of chicks to associate preferably with a familiar rather than an unfamiliar human. This characteristic suggests that ostriches are able to distinguish between humans and adjust their response accordingly. Therefore, handling of ostriches may be facilitated by a familiar/preferred human. In contrast, the arena tests for docility and fear evaluation appeared not to stimulate any bird characteristics which could be link to early habituation to human involvement. As ostriches are flock animals, the tests might have induced stress and discomfort, as a result of isolation from the peers. Also, the behaviour may have been altered due to visual contact with their peers during the tests. The success of containing birds in the marked square during the docility test varied between years and could partly be explained by the difference in experience of handlers between the two years. These results suggest that stockpeople's experience and attitude may have had an important effect on the handling and management of the birds. Hence, appropriate training of stockpeople may be beneficial in improving ostrich welfare and management.

### 6.4 Meat quality and skin damage

Ostriches destined for slaughter may experience stress during transportation or when exposed to a novel environment (such as at the abattoir) or unfamiliar persons, which may result in injuries and/or poorer quality of meat and skin. Habituation to human presence and

interactions has been revealed to reduce stress related to various animal management practices and improve production. In this study, there was no effect of husbandry practices on meat pH, meat colour, carcass attributes and skin damage. It thus seemed that qualitative and quantitative measures of product output were unaffected by the husbandry practices the experimental units were exposed to.

### 6.5 Egg production performance

There is a contention that ostriches habituated to human presence may have their reproduction success compromised as they would direct their sexual behaviour towards humans rather than to their mates. However, female ostriches exposed to such husbandry practices produced more eggs during the first year of entering the breeding flock, than females that received limited human presence and care. There was no evidence of similar benefits in later years, after the birds had been accustomed to the pair-breeding environment. This result suggests that adaptation of females to pair-breeding may be facilitated by early habituation to human presence and positive interactions, and contradicts previous contentions that tamed birds will not reproduce optimally.

### 6.6 Future work

This study has revealed that there is still ample scope for further investigations of factors that could influence ostrich welfare and production. Research priorities would include establishing an acceptable stocking density for rearing chicks when using suggested husbandry practices in this study. Also, the optimisation for docility and fear responses measures with a greater numbers of chicks, as well as the development of an ostrich welfare assessment index need to be considered. Education of stockpeople and the improvement of on-farm management as well as the evaluation of the economic effectiveness of improving animal welfare towards a sustainable ostrich production protocol have to be addressed.

Firstly, considering the size of commercial ostrich farms, the group of chicks exposed to extensive human presence and regular gentle handling in this study may be too small and not representative of the true number of chicks reared by commercial ostrich producers. Therefore, there is a need to establish a stocking density for ostrich chicks that would make the integration of extensive human presence and regular gentle handling feasible.

Secondly, the docility and fear test procedures for ostriches still need to be optimised in order to identify and select breeder birds that are easier to handle and less fearful of humans. Other tests (such as the 'flight distance' or 'novel objects' tests) should also be considered as alternative tests to evaluate fear/boldness of birds towards humans and/or novel situations. It is also important to accumulate data to determine if the reaction of ostriches to the contrived situations during these tests has a genetic basis (hence would respond to genetic selection).

Thirdly, the difference in the success rate of performing the docility test between the two years in this study suggested that handler's experience, attitude, knowledge, and work ethics may also have played an important role in the handling and management of juvenile/adult ostriches. Animal handlers with a positive attitude may provide the best care to animals even if the environment is not conducive thereto. Handlers with a negative attitude and little experience may treat animals poorly even when their environment is favourable. This could ultimately impair the occupational health and safety of the handlers, as well as the welfare of the birds. Thus, the ostrich industry should engage in training competent and confident handlers, establish continuous supervision and in-depth selection of stockpeople based on strong work ethics to promote positivity towards improving the ostrich welfare.

Fourthly, there is a growing concern with regard to food animal welfare by the public worldwide and more specifically in developed countries (Miele et al., 2013). Consumers have

shown a strong willingness to pay more for products derived from animals that received a quality life (Miranda-de la Lama et al., 2017). While South Africa is classified as a developing country and a leader in ostrich production worldwide, its market is based almost entirely on exports to European countries (Brand and Jordaan, 2011). This implies that production of ostriches and product quality should meet the animal welfare standards desired by consumers in the developed European countries. The ostrich industry should thus be transparent to the public on the rearing, management and traceability of ostriches until slaughter. This could be regulated with the establishment of a welfare assessment index and protocol, as developed in other livestock species. The process should be able to stand up to an external auditing process by third party service providers.

Fifthly, while ostrich meat is considered a rich protein source and appears to have gained momentum in the diets of many consumers, diseases such as Avian Influenza threaten the consumption rate and the establishment of a stable market. Applying good animal management practices that improves animal welfare may reduce the probability of disease outbreaks at the farm level. This suggests that the welfare of animals should be the primary element to be evaluated before assessing productivity (Broom, 1997). According to Broom (1997), there are several measures of animal welfare and measuring only one at a particular stage of life does not imply that overall animal welfare is not compromised. Therefore, there is a need to collectively assess the welfare of ostriches throughout their commercial life to establish areas that putatively would need further improvements. To achieve this, association between ostrich producers and professional ostrich research scientists as well as consumers should be encouraged to identify and eliminate barriers for optimum ostrich welfare and production.

Finally, a portion of the world population still live in poverty and lack sufficient protein and energy supplied by their diets (Food and Agricultural Organization, 2009). Yet

the current world population (estimated at 7.6 billion people) is expected to rise, reaching an estimated 9.8 billion people by 2050 (Godfray et al., 2010). Hence, the demand for quality nutritional food sources will grow exponentially in the future. It remains a priority for every livestock industry to be able to participate in food supply to maintain sustainable food security worldwide in a sustainable way without negatively affecting the environment. The ostrich industry finds its place to serve in food security because of the protein rich and low cholesterol red meat derived from the birds. However, competition among animal producers for profit may cause producers and industries to overlook animal welfare. It is likely that an animal production unit that does not promote animal welfare may become obsolete and unsustainable due to low levels of production, poor quality products and a lack of adherence to animal welfare policies. Thus, such an animal production unit will not be economically viable in the long run. Based on the results of the current study, it appeared that the application of extensive human presence and regular gentle handling at an early age in ostriches could improve the welfare and productivity. The economic efficiency of husbandry practices that improve ostrich welfare and sustainability thus needs to be investigated. This should then be followed by investigating potential ways to integrate animal welfare, sustainability and economic effectiveness in a single best-practices guide for farmed ostriches.

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